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THE NUTRITIONAL IMPLICATIONS OF DIETARY FIBER FOR SWINE

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE NUTRITIONAL IMPLICATIONS OF DIETARY FIBER FOR SWINE submitted by JOHN JOSEPH KENNELLY in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION.

DEDICATION

To my wife Louise

and to my parents

Patrick and Mary Kennelly

with my gratitude

ABSTRACT

Forty-eight crossbred pigs of average initial weight 21 kg were fed 10% Tower rapeseed meal (RSM) and 10% Candle RSM as partial replacements for soybean meal (SBM). Diets were formulated to be isocaloric. Pigs fed the SBM diet consumed less feed, gained significantly ($P<0.01$) faster and were more efficient at converting feed to gain than those fed the RSM diets. Performance of pigs fed Candle RSM, was not significantly different from that obtained with Tower RSM. In a second experiment dehulled Tower RSM and Tower RSM hulls were mixed in amounts to produce RSM with crude fiber levels of 6.8, 10.8, 13.5 and 15.8%. Rats fed SBM had significantly ($P<0.05$) higher average daily gain (ADG) than those fed Tower or Candle RSM, or diets containing the rapeseed meats. There was no significant ($P<0.05$) difference in ADG, feed intake, feed to gain ratio or thyroid weight of rats fed either Tower or Candle RSM. Feed intake, feed to gain ratio and fecal volatile fatty acid concentrations increased while ADG decreased with increasing level of hulls in simulated RSM diets.

The influence of qualitative and quantitative differences in energy and protein, in diets with similar crude fiber levels, on performance, carcass composition and digestibility coefficients in swine was studied in an experiment in which 72 pigs, average initial weight 22.6 kg, were fed 4 diets. Diet 1, the control, contained 14.1 MJ digestible energy (DE) per kg , 17.1% crude protein (CP) and

4.1% crude fiber (CF). Diets 2, 3 and 4 each contained 22% oat hulls , 9.8, 9.6, and 10.2% CF , had respective energy contents of 12.2, 12.5 and 14.9 MJ DE/Kg and 17.0, 14.4 and 17.3% CP, respectively. Diets were provided ad libitum. In the growing (22-63 kg) period control animals had significantly greater average daily gain (ADG) than those fed any of the high fiber diets which did not differ significantly. In the finishing (63-92 kg) period there were no significant differences between diets in ADG despite differences in CF intakes ranging from 135.6 to 404.5 g/day. However differences in energy and protein levels in diets with equal CF levels, were shown to affect CF intake, feed intake and feed/gain significantly in both periods. The inclusion of 22% oat hulls in diets 2, 3 and 4 had no significant influence on total backfat. The method of addition of CF significantly influenced dry matter (DM) digestibility with coefficients of 70.2, 72.8 and 65.0% for diets 2, 3 and 4 respectively. Nitrogen digestibility was unaffected by the level of CF in the diet which also failed to alter amino acid digestibility. The method of addition of fiber resulted in significant differences in the digestiblility of CF, neutral detergent fiber and acid detergent fiber in diets with similar CF levels.

Volatile fatty acid (VFA) production in the hindgut of swine was determined using a continuous caecal isotope infusion system. In experiment 1 a control diet (diet 1) containing 4.8% crude fiber (CF) was fed to four pigs fitted

with caecal cannulae. In experiment 2 the same control diet plus two diets (diets 2 and 3) containing, respectively, 9.9 and 15.0% CF were fed. Following a 15 wk adaptation to diet 3 VFA production rates were again determined (experiment 3). In experiment 1 average VFA concentrations, in caecal fluid, were 79.1, 33.0 and 9.9 mmolar while VFA molar percent were 64.8, 27.1 and 8.1 for acetate, propionate and butyrate, respectively. Net production rates for acetate, propionate and butyrate respectively were 42.6, 14.3 and 4.9 mmoles/h. The average contribution of VFA to the maintenance energy requirment of the pig was calculated as 19.7%. In experiment 2 total VFA concentrations for pigs fed diet 1 and 2 were not significantly different. Pigs fed 15% CF had significantly lower total VFA concentrations than those fed diet 2. No significant dietary differences were observed in VFA production rates. However pigs fed the 10% CF diet tended to have highest production rates with intermediate levels being recorded for pigs fed diet 3. The energy contribution of VFA for pigs fed 5, 10 and 15% CF was calculated as 10.1 15.5 and 11.1% of the maintenance energy requirements, respectively. Following a 15 wk adaptation period to the high fiber diets there was no evidence of increased VFA production.

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I. GENERAL INTRODUCTION

Increasing competition between man and animals for low fiber foods and a greater awareness of the role of fiber in human health and disease has resulted in an upsurge of interest in all aspects of dietary fiber by both animal and human nutritionists. In the long term the survival of the pig industry may depend on its ability to utilise high fiber foods for which it does not compete directly with man.

While the principal components of dietary fiber are cellulose, hemicellulose and lignin it also contains highly variable ammounts of pectins, gums and mucilages. Also associated with the fibrous fraction of the diet are waxes, cutins and undigested proteins and minerals. Cellulose consists of an unbranched polymer of D-glucose with B-1,4 linkage. The hemicellulose molecule is usually much smaller than cellulose and encompasses a wide variety of pentosans and hexosans including, for example, xylans and glucomannans. While chemical methods are available to separate the various components of dietary fiber their complexity often precludes their use in routine feed evaluation. The most widely used method for estimating the fiber level of the diet is the well known and aptly named crude fiber (CF) method. The recovery of cellulose, hemicellulose and lignin, respectively, in the CF fraction being 50-80%, approximately 15% and 10-50%. In view of the foregoing, attempts have been made to develop methods which more precisely measure the chemical components of dietary

fiber. In general these methods recognise that fiber gives structural support to the plant and therefore is found primarily in the cell wall. Neutral detergent fiber (NDF) theoretically estimates the total plant cell wall while acid detergent fiber (ADF) recovers both cellulose and lignin. As NDF primarily consists of cellulose, hemicellulose and lignin, NDF minus ADF gives a measure of the hemicellulose content of the diet.

Because the fibrous portions of many feeds may be associated with substances which inhibit animal performance, for example, tannins and glucosinolates, it is extremely difficult to isolate the effect of fiber per se. While rapeseed meal (RSM) contains glucosinolates which have been shown to reduce animal performance, it also contains appreciably higher levels of CF than soybean meal (13% vs 6%). The higher fiber level of RSM has been implicated as a causative agent in the lower performance observed when low glucosinolate RSM is fed as the sole protein supplement in the diets of young pigs. The influence of low fiber varieties of RSM and dehulled RSM, whose CF level was similar to soybean meal, has been examined with animal performance as the criterion. This information should prove useful in predicting the benefits to the pig industry should current efforts to reduce the fiber level of RSM be successful.

The problems associated with current methods of fiber analysis and the confounding which can result from

inhibitory substances associated with the fiber source is not the only dilemma facing the researcher in this area. Having decided on the source and level of dietary fiber, the mode or model to be used in formulating the diet must be selected. While the literature contains a wide range of approaches to this problem most of the studies fall into one of the three categories outlined in Chapter III. Although the fiber level of the diet will be similar regardless of the model chosen, qualitative and quantitative differences in energy and protein will occur. The extent to which these factors modify the effect of fiber on growth and carcass composition has been examined in chapter 3, while their effect on digestibility coefficients has been studied in chapter 4.

Non-ruminant animal research with fiber has been generally of a negative nature. Little attention has been paid to the extent to which swine can utilise various types of fiber. Pigs possess enlarged areas of its gastrointestinal tract where active microbial populations thrive. The principal end products of this microbial fermentation are volatile fatty acids (VFA). While the production rates of VFA and their nutritional and metabolic significance have been studied in depth in ruminants, only limited information is available for swine. In chapter 5, VFA production rates in pigs fitted with caecal cannulas are reported. Isotope dilution techniques with (^{14}C) acetate, (^{14}C) propionate and (^{3}H) butyrate in a continuous infusion

system were used to estimate the effect of fiber, age and/or adaptation of the animal on (a) VFA production rates and (b) their contribution to the maintenance energy requirements of the pig.

In the experiment reported in Chapter V chromic oxide was used as an indicator for the determination of digestibility coefficients. However it was found to be unsatisfactory especially when used in conjunction with grab sampling. The alternative markers which are available are mainly radioactive isotopes which result in animal contamination and problems associated with the disposal of radioactive waste. Instrumental neutron activation analysis (INAA) which allows the use of non radioactive elements as markers overcomes many of these problems. The study outlined in appendix I was designed to develop an INAA method which could be used routinely for the measurement of these markers. The suitability of one of these markers (dysprosium) for determination of digestibility coefficients was examined in the experiment reported in appendix II.

II. THE EFFECTS OF LEVELS OF ISOLATION, OR VARIETAL DIFFERENCES IN, HIGH FIBER HULL FRACTION OF LOW GLUCOSINOLATE RAPESEED MEAL ON RAT AND PIG PERFORMANCE

A. ABSTRACT

Forty-eight crossbred pigs of average initial weight 21 kg were fed 10% Tower rapeseed meal (RSM) and 10% Candle RSM as partial replacements for soybean meal (SBM). Diets were formulated to be isocaloric. Pigs fed the SBM diet consumed less feed, gained significantly ($P<0.01$) faster and were more efficient at converting feed to gain than those fed the RSM diets. Performance of pigs fed Candle RSM, was not significantly different from that obtained with Tower RSM. In a second experiment dehulled Tower RSM and Tower RSM hulls were mixed in amounts to produce RSM with crude fiber levels of 6.8, 10.8, 13.5 and 15.8%. The simulated RSMs and Tower and Candle RSM were used to completely replace SBM in the diets of weanling (75 g) rats. Rats fed SBM had significantly ($P<0.05$) higher average daily gain (ADG) than those fed Tower or Candle RSM, or diets containing the rapeseed meats. There was no significant ($P<0.05$) difference in ADG, feed intake or feed to gain ratio of rats fed either Tower or Candle RSM. Feed intake, feed to gain ratio and fecal volatile fatty acid concentrations increased while ADG decreased with increasing level of hulls in simulated RSM diets. There was no significant difference ($P<0.05$) in thyroid weight between rats fed SBM, Tower RSM or Candle

RSM.

B. INTRODUCTION

Rapeseed meal (RSM) contains a substantially higher crude fiber level than soybean meal (SBM) (13% vs 6%) and this may contribute to the lower performance observed when low glucosinolate RSM is fed as the sole protein supplement in the diets of young pigs (Castell 1977a; Aherne et al. 1977).

The limitations imposed by the high fiber levels of RSM have provided a major incentive for plant breeders to develop varieties of RSM with lower fiber content. Because of the small size of the rapeseed the hull forms a high proportion which makes genetic selection for reduced fiber cultivars difficult (Bayley and Hill 1975). However, Stringam et al. (1974) reported that reduced fiber content in RSM is associated with yellow seedcoat, and that a reduction of about 4% units crude fiber was possible though the introduction of yellow-seeded cultivars.

The objective of experiment 1 was to determine the nutritive value for swine of a recently licensed yellow-coated cultivar of low glucosinolate RSM _Brassica campestris_ (cv Candle). A second experiment was designed to determine the effect of hull level of RSM on the performance of weanling rats. Fecal volatile fatty acid (VFA) concentrations were measured to obtain an estimate of the extent of microbial digestion of the fiber fraction of Tower

RSM (Cummings 1973; Argenzio and Southworth 1974).

C. MATERIALS AND METHODS

Experiment 1

Forty-eight crossbred (Yorkshire x Lacombe) pigs, including equal numbers of barrows and gilts, with an average initial weight of 21 kg were allotted on the basis of sex and weight to the three diets shown in Table 1. The control diet contained SBM as the supplementary protein source, diet 2 contained 10% Candle RSM and diet 3 contained 10% Tower RSM which is a low glucosinolate cultivar of *B. napus* type. The diets, which were calculated to contain 16 % crude protein and to be isocaloric, were based on barley and wheat and formulated to meet or exceed National Academy of Sciences - National Research Council (NAS-NRC 1973) nutrient requirements. The pigs were allowed ad libitum access to feed and water, and the environmental temperature was kept at 21 to 22 °C throughout the experiment. Pigs were housed as mixed sex pairs in 1.2 x 1.2 m partially slotted-floor pens for the duration of the experiment from 20 to 60 kg liveweight.

Data were analyzed using analysis of variance procedures (Steel and Torrie 1960). The two treatment degrees of freedom were partitioned into two orthogonal comparisons: a SBM control vs diets containing RSM comparison and a within RSM comparison.

Experiment 2

Fifty-six male and 14 female weanling Sprague-Dawley rats of average initial weight 75 g were assigned to 10 blocks on the basis of sex and weight. Within each block rats of both sexes were randomly allotted to seven dietary treatments. The composition and chemical analysis of the rat diets are presented in Tables 2 and 3. Tower rapeseed meats and hulls were separated by pin-milling, and were then placed in shallow trays in layers 20 mm deep and autoclaved for 20 min at 100°C to inactivate the myrosinase enzyme (Appelqvist and Josefsson 1967). After autoclaving, the meats and hulls were cooled and dried under vacuum at 37°C. They were then ground and the oil was extracted with hexane during a 24 h period (AOAC 1975). The diets were formulated to be isonitrogenous. Diets 1 through 4 contained Tower rapeseed meats and hulls in ratios to simulate RSM with crude fiber levels of 6.8, 10.8, 13.5 and 15.8%. Tower and Candle RSM were included in diets 5 and 6, and diet 7 contained SBM as the protein supplement. These protein supplements received similar heat treatment to that of the rapeseed meats and hulls.

Rats were chosen for this experiment because of the small amount (10 kg) of dehulled rapeseed available. Rats were individually housed in 24 X 20 X 18 cm stainless steel cages in a room maintained at 22 °C with relative humidity of 50 % and artificial lighting giving 12 h continuous light per 24 h. Feed and water were available ad libitum for the

22-day trial period. Rat weights and feed consumption were recorded weekly.

On day 14 of the experiment rats were continuously observed for an 8 h period, feces were collected as voided, immediately frozen with liquid nitrogen and stored at -20 °C. For VFA analysis feces were thawed, thoroughly mixed and 1 g samples were placed in test tubes. To each tube was added 2 ml distilled water and 0.5 ml of a 50 % phosphoric acid solution in which 1.5 mg cyclohexanone was present as an internal standard. The solution was then homogenized and centrifuged at 8,000 x g for 20 min at 2 °C. The supernatant was then poured off and analyzed for VFA by injecting 0.4 ml into a gas liquid chromatograph (Aerograph 600; D Wilkins Instrument and Research Corp.) with a column packed with 10% SP-1200 (Supelco Inc. Bellefonte, PA. U.S.A.) in 1% phosphoric acid coated on Chromasorb W (80/100 Mesh; Supelco Inc.). The carrier gas was bubbled through distilled formic acid to help minimize ghosting. The response for individual VFA and their molar concentration was calculated by reference to the internal standard and a standard solution (38.2 mmolar acetate, 8.58 mmolar propionate, 4.56 mmolar butyrate and 2.99 mmolar cyclohexanone) injected between duplicate samples. Crude fiber, acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were performed by the standard method of the Association of Official Agricultural Chemists (1975).

On day 22, rats were killed with ether and thyroid

glands were immediately removed and weighed. Data were analyzed using analysis of variance procedures. Treatment means were tested for significant differences using Duncan's Multiple Range test.

D. RESULTS AND DISCUSSION

<u>Experiment 1</u>

An analysis of the RSM used in experiment 1 is included in Table 4. Tower and Candle RSM contained crude fiber levels of 12.9 and 11.2% respectively. ADF (primarily cellulose & lignin) were 25.1% and 18.4%, and NDF (primarily cellulose, hemicellulose and lignin) were 35.5% and 24.3% of Tower and Candle RSM respectively. These results indicate considerable genetic improvement has yet to be achieved before the fiber level of RSM is reduced to that of SBM. The total glucosinolate level of the Candle RSM was 0.84 mg/g and that of the Tower RSM was 0.95 mg/g.

Pigs fed the SBM diet consumed less food, gained significantly ($P<0.01$) faster and were more efficient ($P<0.01$) at converting feed to gain than pigs fed the RSM diets (Table 5). These results are in contrast to those of McKinnon and Bowland (1977) and Moody et al. (1976), but are in agreement with those of Castell (1977b). Differences in protein quality of meal, processing effects, glucosinolate level or level of unprocessed rapeseed contaminating the grain sources might account for the differences reported in these experiments.

Feed intake and performance of pigs fed Candle RSM was not significantly (P<0.05) different from that obtained with pigs fed Tower RSM. Thus, the lower crude fiber, ADF, NDF and glucosinolate levels of Candle were not reflected in significantly improved swine performance.

Experiment 2

Average daily feed intake was least for rats fed the diet containing rapeseed meats alone and increased with increasing levels of added hulls (Table 6). The differences in feed intake were significant (P<0.05) only at the 9.9 and 15.4% inclusion level. The increased intake of these animals may have been a response to higher fiber levels and thus reduced energy content of the diet (Peterson and Baumgardt 1971) or to the dry powdery nature of the meats (Bayley and Hill 1975). A factor contributing to reduced feed intake could be the higher glucosinolate and tannin levels of meats (Seth and Clandinin 1974). Though rats fed SBM had the largest feed intake, the difference was only significantly greater than that of rats fed the diet containing Tower meats alone (diet 1).

Rats fed Tower and Candle RSM had significantly (P<0.05) lower average daily gain (ADG) than those fed SBM. There was no significant difference between rats fed Tower or Candle in average daily feed intake, ADG or feed to gain ratio. This result is similar to that observed with pigs in experiment 1. ADG was significantly greater for rats fed Tower meats (diet 1) than for those fed the highest level of

hulls (diet 4). But, there was no significant difference in ADG of rats fed 5.6, 9.9 or 15.4 % hulls (diets 2, 3 or 4).

Feed to gain ratio was not significantly different between diets containing RSM, SBM or rapeseed meats fed alone. Feed to gain ratio increased as level of hulls in the diet increased (diets 2, 3 and 4) rats fed 5.6 and 9.9% hulls being significantly different from those fed 15.4% hulls. Digestibility coefficients for energy and nitrogen were significantly higher for rats fed diets 2 and 3 than for those fed diet 4 (Table 7). Digestibility coefficients for energy and nitrogen tended to be lower for the Tower and Candle RSM than for the SBM diet. The depressing effect of fiber on digestibility of both nitrogen and energy is in agreement with the observations of other authors (Farrell and Johnson 1972; Teague and Hanson 1954). Digestible energy (DE) and digestible nitrogen (DN) intakes per 100 g gain were lower for rats fed the RSM and SBM diets than for rats fed diets containing rapeseed meats.

No significant differences in thyroid weights of SBM, Tower or Candle fed rats were observed (Table 8). Thyroid weights were significantly ($P<0.05$) heavier for rats fed diets containing rapeseed meats. Thyroid weight increased with increasing level of hulls but the difference was significant only for the diet containing 15.4% hulls, reflecting the higher total percentage of rapeseed in the diet (Table 2).

A significant ($P<0.01$) sex difference in thyroid weight

was observed, with females having more thyroid tissue per 100 g body weight. Male rats fed SBM had significantly lower ($P<0.05$) thyroid weights than males fed Candle whose thyroid weights were not significantly different from those fed Tower RSM. Although not significant, rats fed Candle RSM had consistently higher thyroid weights than those fed Tower RSM. Despite their glucosinolate levels Tower or Candle RSMs fed as sole protein supplement in the diet of rats did not elicit a goitrogenic response.

Table 9 shows increasing fecal VFA concentrations with increasing level of hulls in diets 1 through 4. This is probably a reflection of the increased quantity of undigested material available for microbial fermentation in the caecum and colon as the level of hulls in the diet increased. The concentrations of butyric acid remained relatively more stable, but there were significant ($P<0.01$) increases in both acetic and propionic acid. The higher fecal VFA concentrations recorded in rats fed SBM may be due to the passage of more readily fermentable carbohydrate into the caecum and colon of these rats. A low fiber SBM may also result in a slower rate of passage allowing more time for microbial fermentation. Rats fed Candle (18.4% ADF) had a lower total VFA concentration than rats fed Tower (25.1% ADF). An examination of the ratio of acids show increasing acetate:butyrate and acetate:propionate plus butyrate while acetate:propionate ratio declines. The results indicate that some microbial digestion of rapeseed fiber may occur.

Further study of VFA production rates and absorption are required to determine the extent of this fermentation.

The present experiments indicate that a 2% reduction in the crude fiber level of RSM does not result in improved pig or rat performance. The absence of response in ADG observed in rats following complete removal of hulls indicates that very little of the depression in performance associated with the feeding of RSM is attributable to its high fiber hull fraction. The extent to which other factors which affect palatability are responsible is not clear from the present study. While plant breeders are currently actively pursuing the search for varieties of rapeseed with lower "fiber" levels, results of these experiments makes this an activity of questionable value to the feed industry.

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Table 1. Formulation and composition of diets in experiment 1

Diets Protein Supplement	1 (SBM)	2 (CANDLE RSM)	3 (TOWER RSM)
Barley	40.0	40.0	40.0
Wheat	41.5	39.5	39.5
Rapeseed meal	–	10.0	10.0
Soybean meal	13.7	5.4	5.4
Tallow	1.0	1.3	1.3
Iodized salt	0.4	0.4	0.4
Limestone	1.2	1.2	1.2
Monocalcium phosphate	1.2	1.2	1.2
Mineral & vitamin mix+	1.0	1.0	1.0
	100.0	100.0	100.0
Analysis (%, as-fed)			
Protein	16.2	16.0	16.0
Crude fiber	4.0	4.9	4.9
Lysine(calculated)	0.66	0.64	0.64

+ Contributed the following per kg of diet: Zn, 120 mg; Cu, 10 mg; Mn, 48 mg; Fe, 100 mg; Se, 0.1 mg; Vitamin A, 7,500 IU; Vitamin D, 700 IU; Vitamin E, 45 IU; Riboflavin, 12 mg; Niacin, 40 mg; Calcium pantothenate, 27 mg; Vitamin B12, 28 mcg and .5g Furazolidone.

Table 2. Composition of diets in experiment 2.

Protein Supplement	Tower Meats ‡	Tower Meats + Tower Hulls			Tower RSM	Candle RSM	Soybean Meal
Diet Number	1	2	3	4	5	6	7
Dietary Ingredients							
Barley	69.70	64.85	61.35	56.35	61.90	58.45	69.50
Tower RSM	-	-	-	-	31.5	-	-
Candle RSM	-	-	-	-	-	35.5	-
Tower meat	26.45	26.0	25.5	25.45	-	-	-
Soybean meal	-	-	-	-	-	-	23.75
Tower hulls	-	5.6	9.9	15.4	-	-	-
Salt (iodized)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ground limestone	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mineral premix +	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Corn Oil	1.0	0.7	0.4	-	3.75	3.2	3.9
	100.0	100.0	100.0	100.0	100.0	100.0	100.0

+ Contributed the following per kg of diet: Zn, 120 mg; Cu, 10 mg; Mn, 48 mg; Fe, 100 mg; Se, 0.1 mg; Vitamin A, 7500 IU; Vitamin D, 700 IU; Vitamin E, 45 IU; Riboflavin, 12 mg; Niacin, 40 mg; Calcium pantothenate, 27 mg; Vitamin B12, 28 mcg

‡ Residue remaining after removal of hulls by pin-milling

Table 3. Chemical analysis of diets in experiment 2.

Protein Source	Tower Meats[+]	Tower Meats & Tower Hulls			Tower RSM	Candle RSM	SBM
Treatment Number	1	2	3	4	5	6	7
Protein (Nx6.25)%	19.7	19.8	20.5	20.1	20.0	20.4	20.5
Ether extract, %	5.8	5.9	5.7	5.6	6.0	6.1	5.9
Crude fiber of protein supplement %	6.8	10.8	13.5	15.8	12.9	11.2	6.0
Gross energy, MJ/kg	17.81	18.36	17.67	18.00	17.92	17.85	17.72
Digestible energy, MJ/kg	14.25	13.96	13.31	12.49	13.03	13.33	13.71
Digestible nitrogen, %	13.9	13.1	13.9	12.2	11.9	12.9	13.0

[+] Residue remaining after removal of hulls by pin-milling

Table 4. Composition (%) of rapeseed fractions and meats[+] used in experiment 2

	Tower Meats	Tower Hulls	Tower RSM	Candle RSM
Protein (Nx6.25) (Fat free basis)	47.9	16.4	39.8	37.4
Ash	5.4	4.2	6.4	6.4
Crude fiber	6.8	30.7	12.9	11.2
Acid detergent fiber (ADF)	19.2	63.2	25.1	18.4
Neutral detergent fiber (NDF)	22.7	74.9	35.5	36.9
Hemicellulose (NDF-ADF)	3.5	11.7	10.4	18.5
Cell soluble material	77.3	25.1	64.5	63.1

[+] Residue remaining after removal of hulls by pin-milling.

Table 5. Performance of pigs fed diets containing soybean meal or rapeseed meal: experiment 1

Diets	SBM	10% Candle RSM	10% Tower RSM	Pooled SE	Sig.
Grower period (20-60 kg)					
Number of pigs	16	16	16		
Initial weight,kg	21.6	20.9	20.6	0.29	NS
Final weight, kg	61.0	60.1	60.4	0.39	NS
Daily feed intake, kg	1.98	2.04	2.11	0.06	NS
Daily gain, kg	0.75	0.69	0.67	0.01	**
Feed/gain	2.66	2.98	3.16	0.07	**

** $(P<0.01)$.

Table 6. Feed intake, weight gain, efficiency of feed conversion for rats in experiment 2.

Protein Source	Tower Meats[1]	Tower Meats + 5.6% Hulls[2]	Tower Meats + 9.9% Hulls	Tower Meats + 15.4% Hulls	Tower RSM	Candle RSM	Soybean Meal	SE
Treatment No.	1	2	3	4	5	6	7	
No. of rats	10	10	10	10	10	10	10	
Initial weight, g	75.2	75.1	75.2	74.9	74.4	75.5	74.6	0.35
Final weight gain,g	204.6bc[3]	198.3ab	200.5abc	193.6a	206.7c	209.2c	218.4d	1.12
Daily feed,g	17.6a	18.2ab	18.8b	19.0b	18.2ab	18.0ab	19.1b	0.49
Daily gain, g	5.88bc	5.60ab	5.69abc	5.40a	6.01c	6.08c	6.54d	0.16
Feed/gain	3.07a	3.33b	3.38b	3.61c	3.12a	3.05a	3.01a	0.06

1 Residue remaining after removal of hulls by pin-milling.

2 The hulls which had been removed from Tower RSM by pin-milling.

3 Means in the same row with the same letter are not significantly different ($P<0.05$).

Table 7. Digestibility coefficients and energy and nitrogen to gain ratios obtained with diets fed in experiment 2.

Protein Source	Tower Meats[1]	Tower Meats + 5.6% Hulls[2]	Tower Meats + 9.9% Hulls	Tower Meats + 15.4% Hulls	Tower RSM	Candle RSM	Soybean Meal	SE
Treatment No.	1	2	3	4	5	6	7	
Energy, %	80.0a[3]	76.0b	75.3b	69.4c	72.7b	74.7b	77.4ab	0.91
Nitrogen, %	70.5a	66.2b	67.7b	60.5c	59.3c	63.0bc	63.3bc	0.84
DE: gain, KJ/100g	430.9b	464.4c	451.9c	451.9c	405.8a	405.8a	414.2a	5.65
DN: gain, g/100 g	6.7b	6.8b	7.3b	6.8b	5.8a	6.1a	6.1a	0.08

[1] Residue remaining after removal of hulls by pin-milling.

[2] The hulls which had been removed from Tower RSM by pin-milling.

[3] Means in the same row with the same letter are not significantly different ($P<0.01$)

Table 8. Thyroid weights of rats in experiment 2.

Protein Source	Tower Meats[1]	Tower Meats + 5.6% Hulls[2]	Tower Meats + 9.9% Hulls	Tower Meats + 15.4% Hulls	Tower RSM	Candle RSM	Soybean Meal	SE
Treatment No.	1	2	3	4	5	6	7	
No. of rats	10	10	10	10	10	10	10	
	Thyroid weights mg/100g body weight							
Overall	7.85b[3]	8.57b	8.39b	10.48c	5.85a	6.63a	5.86a	0.4
Male	7.53ab	7.85ab	8.17a	10.18e	5.74cd	6.62bc	5.26d	0.17
Female	9.11ab	11.48b	9.30ab	11.70b	6.27a	6.66a	6.23a	0.3

1 Residue remaining after removal of hulls by pin-milling.

2 The hulls which had been removed from Tower RSM by pin-milling.

3 Means in the same row with the same letter are not significantly different ($P<0.05$).

Table 9. Volatile fatty acid (VFA) concentrations, proportions and ratios of individual fatty acids in rat feces

Protein Source	Tower Meats[1]	Tower Meats ÷ 5.6% Hulls[2]	Tower Meats + 9.9% Hulls	Tower Meats + 15.4% Hulls	Tower RSM	Candle RSM	Soybean Meal	SE
	Average concentrations (mmolar) and proportions of individual VFA							
Acetic (A)	20.6a[3]	27.6b	31.6bc	33.2c	34.3c	31.9bc	31.7bc	0.8
Propionic (P)	3.9a	5.4b	6.2b	6.6b	6.6bc	7.7c	8.5d	0.06
Butyric (B)	7.2a	8.3ab	8.9b	8.9b	12.6c	11.4c	14.9d	0.4
A + P + B	31.7a	41.3b	46.7bc	48.7bc	53.5c	51.0c	55.1c	1.61
A/B	2.86b	3.3bc	3.5bc	3.7c	2.7c	2.8b	2.1a	0.05
A/P	5.3b	5.1b	5.1b	5.0b	5.2b	4.1a	3.7a	0.07
A/P+B	1.86ab	2.0b	2.1bc	2.14bc	1.8b	1.7b	1.4a	0.04

1 Residue remaining after removal of hulls by pin-milling.

2 The hulls which had been removed from Tower RSM by pin-milling.

3 Means in the same row with the same letter are not significantly different ($P<0.01$).

III. THE EFFECT OF FIBER ADDITION TO DIETS FORMULATED TO CONTAIN DIFFERENT LEVELS OF ENERGY AND PROTEIN ON GROWTH AND CARCASS QUALITY OF SWINE

A. ABSTRACT

The influence of dietary crude fiber, and the methods used in formulating high fiber diets, on the performance and carcass composition of swine was studied in an experiment in which 72 pigs, average initial weight 22.6 kg, were fed 4 diets. Diet 1, the control, contained 14.1 MJ digestible energy (DE) per kg , 17.1% crude protein (CP) and 4.1% crude fiber (CF). Diets 2, 3 and 4 each contained 22% oat hulls , 9.8, 9.6, and 10.2% CF , had respective energy contents of 12.2, 12.5 and 14.9 MJ DE/kg and 17.0, 14.4 and 17.3% CP, respectively. Diets were provided ad libitum. In the growing (22-63 kg) period control animals had significantly greater average daily gain (ADG) than those fed any of the high fiber diets which did not differ significantly. In the finishing (63-92 kg) period there were no significant differences between diets in ADG despite differences in CF intakes ranging from 135.6 to 404.5 g/day. However differences in energy and protein levels in diets with equal CF levels, were shown to affect CF intake, feed intake and feed/gain significantly in both periods. The inclusion of 22% oat hulls in diets 2, 3 and 4 had no significant influence on total backfat . The present results indicate that changes in protein and energy associated with fiber

addition to swine diets and the physiological age or adaptation of the animal to the dietary fiber may significantly influence the parameters measured and the conclusions drawn.

B. INTRODUCTION

There is considerable disagreement among authors with respect to the influence of dietary crude fiber on the growth, feed/gain (FG) and carcass characteristics of swine, especially with reference to the effect of fiber on carcass fat content (Crampton et al. 1954a; Merkel et al. 1958a; Pond et al. 1962; Hochstetler et al. 1959; Cunningham et al. 1961; Noland and Scott 1960; Klay et al. 1964; Baird et al. 1969; 1970; 1974; Larsen and Oldfield, 1961; Teague and Hanson 1954). These differences could be due in part to the source of fiber and hence its composition.

Crude fiber determination is a very imprecise analytical procedure. The recovery of cellulose, hemicellulose and lignin in the crude fiber fraction being on average 50-80%, 20% and 10-50% respectively (Van Soest and McQueen 1973). The origin of the cellulose, (Forbes and Hamilton 1952), hemicellulose and lignin (Nehring and Uhlemann 1972), the degree of cross linkage (Mangold 1934) and processing effects (Crampton and Bell 1946, Calder et al. 1959) are further sources of variation in dietary fiber leading to considerable differences in its physical and

chemical composition and hence the biological response of the animal. Comparisons between various studies are further complicated because of differences in protein and energy levels in the diets arising from the dietary model selected. Furthermore, changes in fiber levels are frequently associated and confounded with alteration in fiber and protein source (Bohman et al., 1955).

While the criteria used in formulating high fiber diets vary considerably the majority of studies can be divided into the following broad categories:

1. The high fiber diet is maintained isonitrogenous but no attempt is made to maintain a constant digestible energy (DE) level (Baird et al. 1970; Boenker et al. 1969; Bohman et al. 1955; Hochstetler et al. 1959; Lowrey et al. 1959),

2. The high fiber diet is achieved by a simple dilution of the control diet with the fiber source resulting in decreased energy density, and with protein quality and quantity changing with the protein content of the fiber source (Ademosun 1976; Axelsson and Eriksson 1953; Boenker et al. 1969; Bohman et al. 1953; Coey and Robinson 1954; Corley et al. 1978; Crampton et al. 1954; Cunningham et al. 1961; Farrell and Johnson 1970; Friend et al. 1962; Larsen and Oldfield 1961; Lloyd and Crampton 1955; Lowrey et al. 1959; Merkel et al. 1958a; Pals and Ewan 1978; Stevenson et al. 1959; and Teague and Hanson 1954), and

3. The high fiber diet is formulated to be of a similar nitrogen and digestible energy content as the control diet

(Baird et al. 1969; 1970; 1974; Merkel et al. 1958; Forbes and Hamilton 1952).

The present study was designed to compare high fiber diets formulated according to the above three categories with respect to the effect of dietary fiber on the performance and carcass characteristics of growing-finishing swine. Oat hulls were selected as the fiber source because their low crude protein level (4%) helped prevent confounding of fiber level with source of protein.

C. MATERIALS AND METHODS

Experimental

Three models for determining the effect of dietary fiber on the performance and carcass composition of swine were examined with 72 crossbred (Yorkshire x Lacombe) pigs (38 barrows, 34 gilts) of average initial weight 22.6 kg. Pigs were randomly allotted on the basis of sex and weight to the four diets shown (Table 1). Pigs on treatment 1, were fed a standard University of Alberta swine diet containing 17.1% crude protein (CP) and 4.1% crude fiber (CF). Dietary treaments 2, 3 and 4 were formulated, on the basis of published values (Allen 1978), to contain equal CF levels of 10.0%, the greater portion of the CF arising from the addition of 22% ground oat hulls to each diet . Treatment 2 was formulated to be isonitrogenous with treatment 1 while treatment 4 was both isonitrogenous and of similar

digestible energy with the control diet. Treatment 3 was obtained by a simple dilution of 78 kg control ration with 22 kg oat hulls thereby reducing the CP level of treatment 3 from 17.1 to 14.4%.

Pigs were penned in pairs of the same sex in concrete-floored pens (1.52 x 4.06 m) without bedding. The pigs were allowed ad libitum access to feed and water and the environmental temperature was controlled at 21-23°C throughout the experiment. All diets were fed in meal form and weekly feed intake and pig weights were recorded. At the conclusion of the growing period (63 kg), four pigs from each treatment were transferred to a digestibility trial from which the digestible energy values (Table 1) were determined following a 7-day total fecal collection (Chapter IV). Forty animals continued on experiment until slaughtered at 90-95 kg when carcass measurements and commercial grade indexes were obtained (Table 3). Proximate analyses of the diets (Table 1) were performed by the standard methods of the Association of Official Agricultural Chemists (1975).

Statistical Analysis

All statistical analyses were conducted on an individual pig basis, except feed intake and FG for which results were computed on a pen basis. Data were analyzed using least squares analysis of variance for unequal numbers (Harvey 1960) adjusting for all identified sources of variation. Means for significant treatment differences were compared

using Student-Newman-Keuls multiple range test (Steel and Torrie 1960) taking into account unequal number of observations per mean.

D. RESULTS

Performance data

Growing period (22-63 kg) While pigs tended to increase feed intake when fed low energy diets the differences were not significant. The inclusion of 11.5% tallow in diet 4 was associated with a significant reduction in feed intake from that obtained with pigs fed all other diets. No significant differences were observed, between pigs fed the three high fiber diets in DE intake.

The inclusion of 22% oat hulls caused a significant reduction in average daily gain (ADG) in pigs fed diets 2, 3 and 4 (Table 2). Pigs fed diet 4 had similar feed/gain (FG) to those fed the control diet which was significantly better than that attained by pigs fed diets 2 and 3.

Finishing period (63-92 kg)

The effect of energy level of the diet on feed intake was more apparent in the finishing period because pigs fed diet 2 had significantly higher feed intake than pigs fed diet 1 or 4. While not significant, pigs fed diet 3 also tended to have higher feed intakes resulting in similar DE intakes and the absence of significant treatment difference in ADG between pigs fed the high CF and those fed the control (4.1%

CF) diet. Feed to gain ratio reflected the crude fiber and energy level of the diets with pigs fed diets 1 and 4 having significantly lower FG than those fed diets 2 and 3 which did not differ ($P < 0.05$) from each other.

<u>Overall period (22-92 kg)</u>

Pigs fed the control diet required significantly fewer days to reach slaughter weight than pigs fed the three high fiber diets (Table 2). There were no significant differences in days on test between pigs fed fiber diets formulated to the three models; however, animals fed diet 4 had significantly ($P<0.05$) lower feed intake than those fed diet 2. Feed intake was not significantly different between diets 2 and 3. There was no significant treatment difference in DE intake, but, pigs fed the control diet tended to have higher intake than those fed the high fiber diets. While not significant, feed intake on the high fat diet was depressed, in both growth periods, below that obtained with pigs fed the control diet. Pigs fed diet 4 had significantly lower daily CF intake than those fed diets 2 and 3 which had similar CF levels. Considerable differences in CF intake occurred in the finishing periods between pigs fed diets 2 and 3 with pigs fed diet 3 having significantly lower intake.

<u>Carcass data</u>

A summary of the results of carcass measurements are

presented (Table 3). There were no significant treatment differences in final weight, but there was a significant reduction in carcass weight of pigs fed diet 3 compared to control animals. Animals fed diets 2 and 4 also tended to have lower carcass weights. Therefore, all carcass data presented in Tables 3 and 4 with the exception of dressing percent were adjusted for final carcass weihgt. However, correcting for final carcass weight did not influence treatment differences. Final weight was used as a covariate for dressing percent. However, no significant influence of diet on dressing percentage or total backfat (the sum of three measurements shoulder, back and loin) was observed. Ham weight, area of lean in ham , percent ham of side and predicted percent yield of trimmed cuts (Anonymous 1968) showed no significant differences across treatments. There were no significant treatment differences in predicted percent yield of trimmed cuts, however, pigs fed diet 2 had significantly better commercial grade index than pigs fed diets 1 or 4.

Table 4 contains a summary of the effect of treatment on carcass quality of barrows and gilts. Sex significantly affected backfat, loin area, area of lean in ham,commercial grade index and percent yield of trimmed cuts but had no significant influence on dressing % , ham weight or area of lean in ham. Barrows had more backfat but lower loin area, commercial grade index, and yield of lean cuts than gilts. With the exception of commercial grade index (Table 4) there

were no significant treatment by sex interactions.

E. DISCUSSION

The inclusion of fiber in swine diets decreases energy digestibility and generally results in increased feed intake (Crampton et al. 1954a; Baird et al. 1975; Dinusson et al. 1961; Noland and Scott 1960) as the animal attempts to maintain digestible energy intake (Agricultural Research Council 1967; Baird et al. 1975; National Academy of Sciences-National Research Council 1979). Levels of crude fiber in excess of 10-15% of the diet, depending on the fiber source, processing effects, and the age and/or length of adaptation, frequently result in depressed feed intake. It is important, therefore, to distinguish between the effects of crude fiber resulting from a simple dilution of digestible energy and that due to bulk or palatability (Braude 1967). This is particularly true in the case of alfalfa where increases in crude fiber level following its addition to the diet frequently fails to elicit an increased feed intake and may even result in a depression in feed intake (Bohman et al. 1955; Hanson et al. 1956). It seems likely that differences in relative feed intake observed where similar fiber sources have been studied (Jensen et al. 1959, 1959a), Larsen and Oldfield 1961; Crampton et al. 1954a; Dinusson et al. 1961; Noland and Scott 1960) can be attributed, at least in part, to changes in palatability due

to differences in processing (Calder et al. 1959, Crampton and Bell 1946, Patience et al. 1977).

<u>Dietary fiber and performance</u>

In the growing period (22-63 kg) the energy dilution resulting from the inclusion of 22% oat hulls (diet 2 and 3) had no significant influence on feed intake. Pigs fed high CF diets increased their feed intake in the finishing (63-92 kg) period sufficiently to achieve similar or greater digestible energy intake to pigs fed the control diet. It is not clear whether the increased feed intake of pigs fed diets 2 and 3 in the finishing period was a result of the increased age of the animal or due to animal and/or microbial adaptation to the dietary fiber. It would appear that the duration of the experiment and age of the animal could be important variables in fiber studies and could significantly influence the results obtained.

The availability of feed influences the pigs ability to maintain DE intakes when presented with high fiber diets. Studies conducted where free access to feed is not allowed can lead to erroneous conclusions as limited time feeding inevitably leads to restriction in feed intake (Wyllie and Owen 1978). The influence of restricted feeding in studies where free access to feed is not allowed (Babatunde et al. 1966; 1967; Barber et al. 1972; Calder et al. 1959; Clawson et al. 1962; Coey and Robinson 1954; Crampton et al. 1954,

1954a; Cunningham et al. 1962; Davies and Lucas 1972; Fuller and Livingstone 1978; Jensen et al. 1959a; Lucas and Calder 1956; Vanschoubroek et al. 1967) can thus be confounded with the effect of dietary fiber.

The ADG in the growing period was closely related to digestible energy intake and was significantly greater for the control pigs than for those fed the three high fiber diets. However as the pigs increased in age and/or adapted to the high fiber diet their ability to eat to digestible energy requirements resulted in similar average daily gains to those obtained on the control diet. While there were no significant differences due to the addition of 22% oat hulls in the finishing period, the control animals gained significantly faster overall .

Dietary fiber and carcass quality

The level of crude fiber in the diet did not significantly influence any carcass measurement. However the inclusion of 22% oat hulls in diets 2, 3 and 4 reduced dressing percent by 1.6, 2.1 and 0.7 units respectively. When corrected for carcass weight (Table 3) the situation is reversed with pigs fed high fiber diets having larger dressing percent. Coey and Robinson (1954) suggest that the reduced dressing percent, associated with larger gut fill, contributes at least in part to the lower backfat sometimes observed following addition of fiber to swine diets. The

lower carcass weight of animals fed high fiber diets place them at a younger physiological age and consequently lower carcass fat. However, in the present study backfat was not altered when corrected for carcass weight.

The failure of dietary fiber to significantly reduce carcass fat or increase carcass leanness is in agreement with the results of Hochstetler et al. (1959), Klay et al. (1964), Baird et al. (1969, 1970, 1974), Larsen and Oldfield (1961), Teague and Hanson (1954) but contrary to the reports of Axelsson and Eriksson (1953), Bohman et al. (1955), Crampton et al. (1954), Cunningham et al. (1961), Merkel et al. (1958, 1958a), Pond et al. (1962), Troelsen and Bell (1962), Waldern (1964),

The absence of effect of dietary fiber on carcass fat reported in the above experiments can be explained on the basis of factors other than the CF level of the diet.

The tendency to decreased carcass fat as dietary CF level increased reported by Axelsson and Eriksson (1953) is confounded by the experimental design used. Straw was used as the source of fiber while all animals including the control group were bedded on straw, reported intakes of crude fiber are therefore open to question. Decreased backfat on high fiber diets reported by Bohman et al. (1955), Cunningham et al. (1961), Pond et al. (1962) is not surprising as in contrast to other reports pigs failed to increase feed intake in response to increased dietary crude fiber levels. In the case of Bohman et al. (1955) this

response can be attributed to the inhibitory influence of alfalfa on feed intake (Hanson et al. 1956, Becker et al. 1956). Resulting changes in backfat can therefore be attributed to restriction in energy intake and differences in palatability rather than energy dilution due to level of CF. As feed was not available ad libitum in the studies of Crampton et al. (1954a) and Troelsen and Bell (1962) it is impossible to distinguish between the effect of reduced intake and increased fiber level of the diet.

Highly significant correlation coefficients between percent CF and backfat thickness reported by Merkel et al. (1958, 1958a) are surprising as the authors report no significant influence on backfat with diets varying in CF percent from 3.14 to 13.56. Significantly reduced backfat was only observed following the incorporation of 52% of "extremely weathered" alfalfa hay into the diet with resulting CF level of 19.85%. While Waldern (1964) reported decreased back and loin fat on high fiber diets no significant differences were observed in shoulder fat. It is not clear to what extent results were influenced by confounding changes in source of protein with fiber level.

Differences in carcass traits between barrow and gilts are reported in Table 4. While barrows had significantly lower area of loin, commercial grade index, calculated percent yeild of trimmed cuts and area of lean in ham than gilts, no significant differences were observed in dressing percent or ham weight.

Effect of differences in protein and energy level in high fiber diets

Pigs fed the three high fiber diets differed significantly in feed intake and hence CF intake in the growing and finishing periods. Pigs fed diets with similar CF percent and fiber source showed differences in CF intake of 25.0 g/day and 78.4 g/day for the growing and finishing periods, respectively (Table 2). This emphasizes the need for caution when comparisons are being made, even if the level and source of CF are the same. The influence of CF per se on growth and carcass quality is more likely to be mediated through the actual CF intake rather than the dietary CF percent. Some of the treatment differences in performance and carcass traits, can be attributed to differences in energy (Talley et al. 1976) and protein level, which can have an important influence on the results obtained where diets with similar fiber level and source are used.

Pigs tend to increase feed intake in an attempt to maintain digestible energy intake when presented with increased levels of dietary fiber. The ability of the pig to maintain digestible energy intake appears to be related to the period of adaptation to the diet and/or physiological age of the animal. Failure of swine to maintain digestible energy intake can be attributed to changes in palatability due to excessive levels or inhibitory substances in the fiber source. Where any factor other than fiber per se

renders high fiber diets unpalatable the effect on performance and carcass quality should not be attributed to the energy dilution of the crude fiber fraction of the diet. Many of the results attributed to the energy dilution effect of CF or the effect of CF per se are more likely to be due to changes in palatability of the diet. In this study, pigs achieved similar digestible energy intakes, in the finishing period, on 10% CF diets to that attained on a 4% CF diet. Under such circumstances it was shown that the level of CF had no significant effect on carcass quality. Qualitative and quantitative differences in energy and protein were shown to influence feed intake and CF intake significantly on diets with similar CF levels.

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Table 1. Formulation and composition of experimental diets

Diets	1	2	3	4
Ingredients (% as fed)				
Barley	50.0	35.4	39.0	40.0
Wheat	31.5	20.0	24.5	-
Soybean meal	15.0	19.1	11.7	23.0
Oat hulls	-	22.0	22.0	22.0
Iodized salt	0.5	0.5	0.4	0.5
Calcium phosphate	1.0	1.0	0.8	1.0
Calcium carbonate	1.0	1.0	0.8	1.0
Vit. mineral mix†	1.0	1.0	0.8	1.0
Tallow	-	-	-	11.5
	100.0	100.0	100.0	100.0
Analysis (as-fed)				
Dry matter (%)	86.6	88.8	88.2	85.6
Gross energy (MJ/kg)	17.10	17.00	16.80	20.70
Digestible energy (MJ/kg)	14.10	12.20	12.50	14.70
Protein (%)	17.13	17.00	14.38	17.31
Crude fiber (%)	4.10	9.80	9.60	10.20
Neutral detergent fiber (%)	19.04	29.90	30.00	29.30
Acid detergent fiber (%)	5.40	11.80	10.80	12.40
Ether extract (%)	2.13	1.97	2.02	15.60
Ash (%)	5.32	6.27	5.46	5.46

† Contributed the following per kilogram of diet: Zn, 120 mg; Cu, 10 mg; Mn 48 mg; Fe, 100 mg, Se, 0.1 mg; vitamin A, 7,500 IU; vitamin D, 700 IU; vitamin E, 45 IU; riboflavin, 12 mg; niacin, 40 mg; calcium pantothenate, 27 mg; vitamin B_{12}, 28 μg

Table 2. Feed, digestible energy (DE) and crude fiber (CF) intake, daily gain and feed/gain of pigs from 22 to 63 kg, 63 to 92 kg, and overall

Diet DE (MJ/kg) CF (%)	1 14.1 4.1	2 12.2 9.8	3 12.5 9.6	4 14.7 10.2	S.E.M.
Period 1 (22-63kg)					
No. animals	18	18	18	18	
Initial wt (kg)	22.9	22.7	22.0	22.9	
Daily feed intake (kg)	2.01a	2.19a	2.17a	1.86b	0.19
Daily DE intake (MJ)	28.3	26.7	27.1	27.3	0.07
Daily CF intake (g)	82.4a	214.7b	208.3b	189.7c	0.98
Daily gain (kg)	0.75a	0.68b	0.66b	0.66b	8.10
Feed/gain	2.71a	3.22b	3.31b	2.79a	0.01
					0.08
Period 2 (63-92kg)					
No. animals	† 10	‡10	‡10	†10	
Daily feed intake (kg)	3.32a	4.12b	3.66ab	3.20a	0.16
Daily DE intake (MJ)	46.8	50.3	45.7	47.0	1.90
Daily CF intake (g)	135.60a	404.50b	351.70c	326.10d	13.90
Daily gain (kg)	0.84	0.80	0.78	0.81	0.03
Feed/gain	3.83a	5.22b	5.12b	3.90a	0.11
Overall (22-92kg)					
No. animals	10	10	10	10	
Final wt (kg)	92.3	92.3	91.7	92.1	1.9
Days on test	92.8a	100.3b	103.1b	98.7b	1.7
Daily feed intake (kg)	2.80ab	3.08b	3.00ab	2.52a	0.14
Daily DE intake (MJ)	39.5	37.6	37.5	37.5	1.7
Daily CF intake (g)	115.40a	300.90b	288.50b	257.20c	9.00
Daily gain (kg)	0.81a	0.75b	0.72b	0.75b	0.02
Feed/gain	3.25a	4.14b	4.24b	3.33a	0.07

a,b,c,d means in the same row with the same letter are not different ($P < 0.05$)

† 4 barrows and 6 gilts

‡ 6 barrows and 4 gilts

Table 3. Summary of carcass data

Diets	1	2	3	4	S.E.M.
No. of animals	10	10	10	10	
Days on test	92.8a	100.3b	103.1b	98.7b	1.7
Final wt (kg)	92.3	92.3	91.7	92.1	1.9
Hot carcass wt[§] (kg)	76.4a	73.7ab	72.0b	74.6ab	0.7
Dressing (%)	78.5	76.9	76.4	77.8	1.2
Total backfat[+] (cm)	10.2	9.5	10.1	10.3	0.2
Ham wt (kg)	8.7	8.7	8.7	8.6	0.1
Area of lean in ham (cm^2)	134.2	135.9	131.4	129.5	6.4
Percent ham of side (%)	27.0	27.1	26.9	26.7	0.4
Area of loin (cm^2)	29.7	31.6	30.8	31.0	1.2
Commercial grade index	101.6ac	103.2b	102.3bc	100.3a	0.6
ROP score[‡]	69.1	70.5	69.3	68.6	0.8

§ all carcass data except dressing percent adjusted using carcass weight as a covariate

\+ sum of three measurements (shoulder, back and loin)

‡ predicted percent yield of trimmed cuts.

a,b means in the same row with the same letter are not different ($P<0.05$)

Table 4. Effect of treatment on carcass quality of barrows (M) and gilts (F)

	Diet	1	2	3	4	S.E.M.	Mean [+]
Hot carcass wt [Ω] (kg)	M	77.1	75.2	72.2	76.4	1.0	75.2*
	F	75.7	72.2	71.8	72.9	1.0	73.2
Dressing (%)	M	78.4	77.3	76.9	78.4	1.7	77.8*
	F	78.6	76.5	75.9	77.1	1.7	77.0
Total backfat‡ (cm)	M	10.3	10.0	10.3	10.8	0.3	10.4*
	F	10.0	8.9	9.8	9.8	0.3	9.7
Area of loin (cm^2)	M	28.9	29.3	29.6	28.4	1.6	29.0*
	F	30.5	34.0	32.0	33.7	1.6	32.5
Ham wt (kg)	M	8.9	8.5	8.5	8.4	0.2	8.6
	F	8.6	8.8	8.8	8.8	0.2	8.8
Area of lean in ham (cm^2)	M	130.4	134.9	126.7	112.6	1.3	126.1*
	F	137.9	136.9	136.1	146.4	1.3	139.3
Commercial grade index	M	101.3a	101.8ab	102.1ab	97.7b	0.9	100.7*
	F	101.8a	104.6b	102.5ab	102.9ab	0.9	103.0
ROP score [§]	M	68.2	69.6	69.1	66.1	1.1	68.2*
	F	70.1	71.4	69.5	70.1	1.1	70.5

+ mean for M significantly different (* $P<0.05$) to F mean for same measurement
Ω all measurements except dressing percent adjusted using carcass weight as a covariate
‡ sum of three measurements (shoulder, back and loin)
§ predicted percent yield of trimmed cuts.
a,b means in the same row with same letter are not different ($P< 0.05$)

IV. THE EFFECT OF FIBER IN DIETS FORMULATED TO CONTAIN DIFFERENT LEVELS OF ENERGY AND PROTEIN ON DIGESTIBILITY COEFFICIENTS IN SWINE

A. ABSTRACT

The influence of dietary crude fiber (CF), and the methods used in formulating high fiber diets, on digestibility coefficients has been investigated. Following a 10 week adaptation period to the four dietary treatments, 2 barrows and 2 gilts ,per treatment, of an average initial weight of 67kg, were transferred to metabolism crates. Diet 1, contained 14.1 MJ digestible energy (DE) per kg, 17.1% crude protein (CP) and 4.1% crude fiber (CF). Diet 2, 3 and 4 each contained 22% oats hulls, which were added to the diets isonitrogenously (diet 2), by simple dilution (diet 3), or isonitrogenously and isoenergetically (diet 4). The three diets contained, respectively, 9.8, 9.6 and 10.2% CF, 12.2, 12.5 and 14.9 MJ DE/kg and 17.0, 14.4 and 17.3% CP. The method of addition of CF significantly influenced dry matter (DM) digestibility with coefficients of 70.2, 72.8 and 65.0% for diets 2, 3 and 4 respectively. The addition of 11.5% tallow to diet 4 significantly improved ether extract digestibility over that obtained with diets 1, 2 and 3. Nitrogen digestibility was unaffected by the level of CF in the diet which also failed to alter amino acid digestibility. The method of addition of fiber resulted in significant differences in the digestiblility of CF, neutral

detergent fiber and acid detergent fiber in diets with similar CF levels.

B. INTRODUCTION

Attempts to isolate the influence of crude fiber on digestibility coefficients from the effects of resulting concurrent qualitative and quantitative changes in other dietary constituents has led to the use of diets in research which though equal in crude fiber levels differ markedly in other respects (Kennelly and Aherne 1980)

The effect of crude fiber is frequently confounded with qualitative and quantitative changes in protein and energy level (Lloyd and Crampton 1955; Greeley et al. 1964) and may be further complicated by differences in palatability associated with the use of a particular fiber source (Larsen and Oldfield 1961).

Utilization of crude fiber by nonruminants has been shown to vary considerably depending on the fiber source (Mangold 1934; Breirem et al. 1958; Bell 1960; Nehring and Uhlemann 1972), degree of lignification (Forbes and Hamilton 1952), level of inclusion (Just 1979; Farrell and Johnson 1970) and the extent of processing (Crampton and Bell 1946; Saunders et al. 1969; McNabb 1975). The results obtained and the conclusions drawn will also be influenced by the physical and chemical composition of the total diet (Schneider and Lucas 1950; Myer et al. 1975), level of

feeding (Cunningham et al. 1962), age and weight of the animal (Nordfeldt et al. 1954; Zivokovic and Bowland 1970), adaptation to the fiber source (Pollman et al. 1979), individual differences among animals (Whiting and Bezeau 1957; Spiller and Shipley 1977) and in their microbial flora (Forbes and Hamilton 1952; Friend et al 1963). Furthermore, crude fiber represents only part of the fiber intake of the animal (Van Soest and McQueen 1973). It is not a discrete chemical entity (Southgate 1973) and may contain highly variable proportions of cellulose, hemicellulose and lignin (Van Soest and McQueen 1973; Kennelly and Aherne 1980).

In view of the foregoing it is hardly surprising that the digestibility of crude fiber has been shown to vary between zero and 97% (Rerat 1978) and that the literature contains conflicting reports on the effects of crude fiber on the digestibility of other dietary nutrients.

The objectives of the present study were to examine the influence on digestibility coefficients of qualitative and quantitative differences in protein and energy levels associated with methods which are frequently used to add fiber to swine diets (Kennelly and Aherne 1980).

C. MATERIALS AND METHODS

Experimental

The effect of qualitative and quantitative differences in energy and nitrogen levels in high fiber diets on

digestibility coefficients were determined with 16 pigs which had been fed diets of the same composition as those used in the present study for a period of 10-weeks from 23 kg liveweight (Kennelly and Aherne 1980). Pigs allotted to diet 1 were fed a standard University of Alberta diet containing 2.74% nitrogen and 4.1% crude fiber (CF) (Table 1). Diets 2,3 and 4 were formulated on the basis of published values (Allen 1978), to contain equal CF levels of 10.0%: most of the CF arising from the addition of 22% oat hulls to each diet. Diet 2 was formulated to be isonitrogenous to the control diet. Diet 3 was obtained by a simple dilution of 78 kg control diet with 22 kg oat hulls thereby reducing the nitrogen level in diet 3 from 2.74 to 2.30%. Diet 4 was both isonitrogenous with and had similar DE to the control diet. Equal numbers of barrows and gilts (Yorkshire x Lacombe), were held in stainless steel metabolism crates which permitted separate collection of urine and feces. The environmental temperature was maintained at approximately 20°C. The average weight of the pigs was 67 kg at the start and 75 kg at the end of the experiment. The animals were allowed 7 days to adjust to the feeding procedures and metabolism crates prior to the commencement of a 7-day total fecal collection period. Urine was collected on days 4-7 inclusive of the total fecal collection period. Female pigs were fitted with bladder catheters for urine collection while in the case of male animals the design of the crates kept contamination of urine

by fecal material to a minimum. The pH of the urine was maintained acidic during collections by the presence of HCl in the holding vessels. Because of limitations in the number of metabolism crates available, the experiment was conducted in 2 phases with 2 pigs per treatment in each phase. Pigs were fed 0.7 kg at 0800 h and 1.4 kg at 1600 h of the diets shown in Table 1. Water was available ad libitum. The experimental diets were sampled as fed. Composite samples were ground through a 20 mesh screen prior to analysis. Total fecal collections from each animal were collected once daily and thoroughly mixed prior to drying in a forced air oven at 60°C for 3 days to determine dry matter. Samples from dried daily fecal collections were then ground through a 20 mesh screen and proximate analyses (Table 1) were performed by the standard methods of the Association of Offical Agricultural Chemists (1975). Amino acid analyses (Table 2) were by the procedures described by Sarwar and Bowland (1975). Acid detergent fiber (ADF) analyses (Van Soest 1963) and neutral detergent fiber (NDF) analyses (Van Soest and Wine 1967), using a pre-digestion step for feed samples (McQueen and Nicholson 1979) were also performed.

<u>Statistical analysis</u>

All statistical analysis were conducted on an individual pig basis. Data were analyzed using least squares analysis of variance for unequal numbers (Harvey 1960) adjusting for all identified sources of variation . Means

for significant treatment differences were compared using Student - Newman - Keuls multiple range test (Steel and Torrie 1960) with adjustments for unequal number of observations per mean.

D. RESULTS

While formulated to be equal in DE (Allen 1978), analysis of diets and of feces showed DE levels of 14.1 and 14.7 MJ/kg respectively for diets 1 and 4 (Table 1). Diets 2, 3 and 4 formulated to contain 10% CF, were shown to have CF levels of 9.8, 9.6 and 10.2% respectively. Diets 2, 3 and 4 were significantly lower in gross energy (GE) digestibility than diet 1 (Table 3). As was required by the design of the experiment, DE intake of pigs fed diets 1 and 4 were the same and both groups had significantly greater DE intakes than pigs fed diets 2 and 3. While DM intake was similar for all experimental diets, considerable differences were observed in DM excretion. The inclusion of 10% CF (diets 2, 3 and 4) caused a significant reduction in dry matter digestibility (DMD) over that obtained with pigs fed the control (4.1% CF) diet (Table 3). This reduction was most marked in the case of pigs fed diet 4 whose DMD was 65.0% compared with 81.4% for pigs fed the low fiber control diet. Dry matter digestibilities for pigs fed diets 2 and 3, at 70.2 and 72.8% respectively, were not significantly different from each other but differed markedly ($P<0.01$)

from diets 1 and 4.

The addition of 11.5% tallow to diet 4 increased ether extract (EE) excretion but improved ($P<0.01$) EE digestibility by 16.3, 15.7 and 18.1 percentage units over that obtained for diets 1, 2 and 3 respectively.

Nitrogen

Differences in energy level and protein level and quality in diets with similar CF levels had a significant influence on nitrogen intake, fecal nitrogen and nitrogen absorption but had no influence ($P<0.05$) on nitrogen excretion, digestibility and retention or apparent biological value (Table 4). As DM intakes were similar across treatments the lower percent nitrogen in diet 3 resulted in decreased ($P<0.05$) nitrogen intake. The higher DM excretion observed with pigs fed diets 2, 3 and 4 than those fed the control diet was associated with a lower fecal nitrogen content. The overall result was an absence of significant treatment effect on nitrogen excretion.

Nitrogen digestibility was similar for pigs fed the 4 diets despite differences in the ratio of grain to soybean meal in these diets or the addition of 22% oat hulls to diets 2, 3 and 4. Similarly, these differences had no significant influence on nitrogen retention or biological value. However, pigs fed the low nitrogen diet (diet 3) tended to have lower nitrogen retention and biological value.

Amino acid digestibilities

With the exception of proline (diet 1 vs diet 2) the fiber level of the diet did not exert a detrimental effect on amino acid digestibility (AAD). The differences observed in AAD appear to be more closely related to qualitative and quantitative differences in nitrogen and energy levels rather than the level of CF in the diet. Digestibilities of isoleucine, leucine, lysine, threonine, aspartic acid and serine for pigs fed the high fat diet (diet 4) were significantly higher than those observed for the other 3 diets. These results are in keeping with the higher inclusion level of soybean meal in diet 4. With the exception of proline no significant differences in AAD were observed between diets 2 and 3.

Fiber digestibility

Despite similar CF intakes by pigs fed the 3 high fiber diets, significant differences were observed in both NDF and ADF intake (Table 5). Digestibility coefficients for CF, NDF and ADF tended to be higher for pigs fed the low fiber diet than for those fed the high fiber diets. The addition of a high level of fat (diet 4) depressed the digestibility of CF, NDF and ADF, the differences being significant for CF and NDF. While fiber digestion coefficients observed with pigs fed diet 3 were consistantly larger than those fed diet 2 the differences were only significant for NDF.

E. DISCUSSION

The method of addition of fiber to the diet may exert a considerable influence on the composition of the diet (Table 1). Problems of interpretation of data can also occur within and between experiments because of the inherent limitations of terms like isonitrogenous and isoenergetic. Formulating diets to be isonitrogenous or isoenergetic provides limited information unless the sources and digestibility of nitrogen and energy are specified. This is clearly demonstrated by reference to Table 2 where considerable differences are observed in the amino acid composition of diets 1 and 4 despite the similarity in their nitrogen content.

Depression in GE and DM digestibility observed with increasing dietary fiber level (Table 3) confirm the results of De Goey and Ewan (1975), Forbes and Hamilton (1952) Kennelly et al. (1978) and others. However, the method of addition of CF also exerted a marked influence on DM excretions despite similar DM intakes. Dry matter excretion for diets 2, 3 and 4 were significantly different at 555, 503 and 627 g/day respectively. While the level of CF per se, despite its inherent variability, will almost always have a negative influence on GE and DM digestibility it is unlikely that this relationship will be a constant one. The strong influence of the criteria used in formulating high fiber diets further emphasize the futility of finding a universally applicable correlation. It is hardly surprising,

therefore, that disagreement exists (Nordfeldt et al. 1954) on a general formula to explain the influence of dietary CF on DM digestibility.

The significant improvement in EE digestibility observed with increasing level of EE (Table 3) is in agreement with the work of Greeley et al. (1964) and Boenker et al. (1969).

Nitrogen digestibilities, in diets ranging in CF levels between 4.1 and 10.2%, were not significantly different. These results are in agreement with Gouwens (1966), Friend (1970) and Eggum (1973) but contrary to the reports of Pond et al. (1962), Boenker et al. (1969), Kornegay (1973) and Pals and Ewan (1978).

While the apparent absence of influence of dietary fiber on nitrogen digestibility is confirmed by reference to the AAD (Table 4) it must be recognised that fecal estimates of AAD are susceptible to modification by microbial activity in the hindgut (Mason and Palmer 1973; Mason et al. 1976). With the exception of proline AAD were as high if not higher for pigs fed the high fiber diets as for those fed the control diet. Similarly dietary CF had no significant influence on nitrogen retention or apparent biological value.

The reasons for disagreement among authors with respect to the influence of CF on nitrogen digestibility are probably as varied as the source of fiber and the experimental conditions employed. The reported negative

influence of dietary fiber on nitrogen digestibility can in many cases be attributed to the lower availability of the protein added with the fiber source (Eggum 1973). Thus, where alfalfa is used as a source of fiber it is difficult, if not impossible, to distinguish between the effect of changing protein source and dietary fiber level. In general, where the fiber source does not contribute a significant amount of the dietary protein the effect on protein digestibility is likely to be negligible. Whiting and Bezeau (1957) reported increased metabolic fecal nitrogen excretion in response to increased dietary fiber. Gouwens (1966) fed diets with 7, 14 and 21% CF to swine and concluded that CF had no influence on total nitrogen or individual amino acid excretion. The present results, observed following a 10 week adaptation period to the diet, appear to confirm the report of Gouwens (1966). Because cellulose digestion is dependent on microbial action it is likely that the extent of its digestion will be influenced by the length of time allowed for microbial adaptation. The normal 7-day adaptation period to the diet may not be sufficient to allow for the development of an optimum microbial population. The presence of an active microbial population in the hindgut can also modify or mask changes in nitrogen digestibility. Infusion of cellulose into the caecum of sheep (Mason et al. 1977) resulted in lower urinary nitrogen excretion due to increased bacterial nitrogen assimilation in the hind gut. While increased dietary fiber results in greater quantities

of carbohydrates reaching the hind gut, increased fecal nitrogen of bacterial origin will only arise where the fiber is susceptible to bacterial fermentation. Fiber sources which undergo extensive degradation in the large intestine can, therefore, decrease the apparent digestibility of nitrogen. The possibility of adaptation to a high fiber diet is a further variable which could alter the effect of CF on digestibility coefficients. However Cunningham et al. (1962) concluded that the period of adaptation had little influence on the pig's ability to digest cellulose.

The difficulties associated with obtaining a good measure of dietary fiber intake is illustrated by reference to Table 5. While CF intakes were similar for pigs fed the three high fiber diets, significant differences were observed in NDF and ADF intake. Pigs fed diet 4 had largest CF intake but their NDF intake was significantly lower than observed for pigs fed diets 2 and 3. In theory NDF and ADF should give the best estimate of dietary fiber, however problems associated with their analysis (McQueen and Nicholson 1979) cannot be ignored. The use of CF remains a popular method for estimating dietary fiber primarily due to the absence of a reliable alternative method which can give similar precision and ease of operation. The use of NDF in the analysis of high energy foods is beset by considerable filtering problems. While these problems can be partly alleviated by pepsin and amylase digestion they continue to result in substantial loss of precision. While the use of

ADF and NDF appear more desirable from a chemical standpoint they are unlikely to gain in popularity until problems associated with their analysis are resolved. A chemical and nutritional evaluation of currently used methods for estimating dietary fiber is required to clearly establish their usefulness in feed evaluation.

Despite its aesthetic appeal, the temptation to ascribe a general cause and effect relationship between CF and digestibility coefficients must be resisted. The present results indicate that the dietary model selected in diets with similar CF levels is an important additional variable which can confound the results obtained. The use of a general relationship between CF and digestibility coefficients is of doubtful value and as such is unlikely to have general application.

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Table 1. Formulation, composition and proximate analysis of experimental diets

Diet No.	(1)	(2)	(3)	(4)
Diet	Control	Isonitrogenous	Simple Dilution	Isonitrogenous and Isoenergetic
Ingredients (%, as fed)				
Barley	50.0	35.4	39.0	40.0
Wheat	31.5	20.0	24.5	-
Soybean meal (48% CP)	15.0	19.1	11.7	23.0
Oat hulls	-	22.0	22.0	22.0
Iodized salt	0.5	0.5	0.4	0.5
Calcium phosphate‡	1.0	1.0	0.8	1.0
Calcium carbonate (38% Ca)	1.0	1.0	0.8	1.0
Vit. mineral mix+	1.0	1.0	0.8	1.0
Tallow	-	-	-	11.5
	100.0	100.0	100.0	100.0
Analyses (as-fed)				
Dry matter, (%)	86.6	88.8	88.2	85.6
Gross energy (MJ/kg)	17.10	17.00	16.80	20.70
Digestible energy (MJ/kg)	14.10	12.20	12.50	14.70
Nitrogen (%)	2.74	2.72	2.30	2.77
Crude fiber (%)	4.10	9.80	9.60	10.20
Neutral detergent fiber (%)	19.04	29.90	30.00	29.30
Acid detergent fiber (%)	5.40	11.80	10.80	12.40
Ether extract (%)	2.13	1.97	2.02	15.60
Ash (%)	5.32	6.27	5.46	5.46

+ Contributed the following per kilogram of diet: Zn, 120 mg; Cu, 10 mg; Mn 48 mg; Fe, 100 mg Se, 0.1 mg; vitamin A, 7,500 IU; vitamin D, 700 IU; vitamin E, 45 IU; riboflavin, 12 mg; niacin, 40 mg; calcium pantothenate, 27 mg; vitamin B_{12}, 28µg

‡ 15-18.5% Ca and 20.5% P

Table 2. Amino acid composition of experimental diets.

Diet No. Diet	(1) Control	(2) Isonitrogenous	(3) Simple Dilution	(4) Isonitrogenous and Isoenergetic
Amino Acids (%)				
Indispensable				
Arginine	0.94	0.97	0.70	0.86
Histidine	0.38	0.38	0.33	0.39
Isoleucine	0.64	0.63	0.57	0.60
Leucine	1.15	1.10	1.04	1.05
Lysine	0.77	0.80	0.65	0.84
Methionine	0.24	0.22	0.22	0.19
Phenylalanine	0.81	0.75	0.71	0.73
Threonine	0.57	0.57	0.52	0.54
Valine	0.77	0.72	0.69	0.69
Dispensable				
Alanine	0.63	0.69	0.64	0.57
Aspartic acid	1.30	1.36	1.17	1.33
Glutamic acid	3.51	3.24	3.12	2.81
Glycine	0.63	0.67	0.62	0.56
Proline	1.33	1.11	1.20	1.14
Serine	0.74	0.73	0.67	0.68
Tyrosine	0.38	0.35	0.33	0.32

Table 3. Daily gross energy, dry matter and ether extract intake, digestibility and digestible energy intake of swine fed four diets.

Diet No. Diet	(1) Control	(2) Isonitrogenous	(3) Simple Dilution	(4) Isonitrogenous and Isoenergetic	SEM
GE intake (MJ/day)	35.4a	35.7a	35.3a	42.2b	0.44
GE digestibility (%)	82.4a	71.9b	74.6b	71.0b	0.91
DE intake (MJ/day)	29.2a	25.7b	26.3b	30.0a	0.34
DM intake (g/day)	1819.0	1865.0	1852.0	1798.0	20.40
DM digestibility (%)	81.4a	70.2b	72.8b	65.0c	0.90
EE intake (g/day)	44.7a	41.4a	42.4a	319.3b	9.84
EE digestibility (%)	78.0a	78.6a	76.2a	94.3b	2.10

a,b,c means in the same row with different letters are different ($P<0.05$)

Table 4. Daily nitrogen intake, excretion, digestiblity, retention, apparent biological value and amino acid digestiblities of swine fed four diets.

Diet	Control	Isonitrogenous	Simple Dilution	Isonitrogenous and Isoenergetic	
Diet No.	(1)	(2)	(3)	(4)	SEM
Nitrogen intake (g/day)	56.8a	57.1a	48.4b	56.3a	0.90
Fecal nitrogen (%)	3.0a	1.8b	2.0b	1.6b	0.08
Nitrogen excretion(g/day)	10.2	9.9	10.1	10.2	0.61
Nitrogen digestibility(%)	82.0	82.7	79.1	81.7	1.10
Nitrogen absorbed(g/day)	46.7a	47.2a	38.3b	46.1a	1.20
Nitrogen retained (g/day)	21.0	23.6	14.7	21.6	3.30
Apparent biological value	44.4	49.9	40.0	44.3	6.90
Amino acids digestibilities (%)					
Indispensable					
Arginine	90.9	90.9	89.8	93.7	1.12
Histidine	91.0	89.1	91.0	93.6	1.40
Isoleucine	82.6a	82.1a	82.2a	86.6b	1.14
Leucine	84.7a	84.0a	84.6a	88.1b	1.12
Lysine	82.8a	82.0a	82.8a	89.3b	1.40
Methionine	78.6	76.7	78.9	79.9	1.22
Phenylalanine	86.6	85.6	86.5	89.5	1.12
Threonine	81.1a	80.3a	81.8a	85.8b	1.16
Valine	82.4	81.1	82.0	85.4	1.27
Dispensable					
Alanine	72.8	73.5	74.9	78.0	1.17
Aspartic acid	83.3a	83.3a	83.8a	88.4b	1.13
Glutamic acid	91.8ab	90.4a	92.2ab	93.1b	1.07
Glycine	80.8	80.8	83.2	84.8	1.14
Proline	91.8a	89.1b	92.3a	93.8a	1.17
Serine	86.7a	86.4a	87.3a	90.0b	1.11
Tyrosine	84.9	84.6	84.2	88.8	1.13

a,b means in the same row with different letters are different ($P<0.05$)

Table 5. Daily crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF), intake and digestibility

Diet No.	(1)	(2)	(3)	(4)	
Diet	Control	Isonitrogenous	Simple Dilution	Isonitrogenous and Isoenergetic	
CF intake (g/day)	85.0a	205.8b	201.6b	214.2b	2.12
CF digestibility (%)	27.7a	20.8a	24.9a	14.7b	1.90
NDF intake (g/day)	394.8a	627.9b	630.0b	615.3c	4.75
NDF digestibility (%)	57.8a	39.5b	46.7c	32.6d	1.30
ADF intake (g/day)	112.0a	247.8b	226.8c	260.4b	2.25
ADF digestibility (%)	20.7a	13.9ab	17.0a	8.5b	2.10

a,b,c,d means in the same row with different letters are different ($P<0.05$)

V. PRODUCTION RATES OF ACETIC, PROPIONIC, AND BUTYRIC ACIDS IN SWINE

A. ABSTRACT

Volatile fatty acid (VFA) production in the hindgut of swine was determined using a continuous caecal isotope infusion system. In experiment 1 a control diet (diet 1) containing 12.9 MJ digestible energy (DE) per kg, 2.3% nitrogen and 4.8% crude fiber (CF) was fed to four pigs fitted with caecal cannulae. In experiment 2 the same control diet plus two diets (diets 2 and 3) containing, respectively, 27.3 and 52.0% alfalfa, 10.9 and 9.1 MJ DE, 9.9 and 15.0% CF were fed. In experiment 1 pigs were fed 2.7 kg daily of diet 1 in 24 equal feeds. In experiment 2 pigs were fed 2.4 kg daily of the 3 diets (incomplete block design) in three equal feeds at 8 h intervals. Following a 15 wk adaptation to diet 3 VFA production rates were again determined (experiment 3). In experiment 1 average VFA concentrations, in caecal fluid, were 79.1, 33.0 and 9.9 mmolar while VFA molar percent were 64.8, 27.1 and 8.1 for acetate, propionate and butyrate, respectively. Net production rates for acetate, propionate and butyrate respectively were 42.6, 14.3 and 4.9 mmoles/h. The average contribution of VFA to the maintenance energy requirment of the pig was calculated as 19.7%. In experiment 2 total VFA concentrations for pigs fed diet 1 and 2 were not significantly different. Pigs fed 15% CF had significantly

lower total VFA concentrations than those fed diet 2. No significant dietary differences were observed in VFA production rates. However pigs fed the 10% CF diet tended to have highest production rates with intermediate levels being recorded for pigs fed diet 3. The energy contribution of VFA for pigs fed 5, 10 and 15% CF was calculated as 10.1 15.5 and 11.1% of the maintenance energy requirements, respectively. Following a 15 wk adaptation period to the high fiber diets there was no evidence of increased VFA production.

B. INTRODUCTION

Most mammalian species possess enlarged areas of their gastro-intestinal tract where microbial digestion occurs (Hintz et al. 1978). Volatile fatty acids (VFA), as the principal end products of microbial fermentation (Elsden et al. 1946) are utilised by ruminant animals with an efficiency comparable to that observed for glucose (Hungate 1966). While VFA are the major end products of carbohydrate digestion in ruminants (Bergman et al. 1965; Leng and Brett 1966; Faichney 1969) significant VFA concentrations have also been found to occur in the large intestines of such diverse species as the pony (Glinsky 1976; Argenzio et al. 1974), porcupine (Johnson and McBee 1960), rabbit (Parker 1976; Parker and McMillan 1976), rat (Kim et al. 1978c; Yang et al. 1969; 1970), and pig (Friend et al. 1962; Imoto and

Namioka 1978a; Kim et al. 1978a)

The microflora found in the hindgut of swine are similar to those observed in the rumen (Salinatro et al. 1977). Furthermore, the caecum and colon of swine have been shown to transport VFA at rates equivalent to or greater than equine large intestinal mucosa or bovine rumen epithelium (Argenzio and Southworth 1974). The large intestine of swine comprises 38% of the volume of the gastrointestinal tract in contrast to 11% in the case of the cow (Bayley 1978). The retention time of digesta in the large intestine of swine is similar to that observed for sheep rumen (Hecker and Grovum 1975; Braude 1976) and 2 and 3 times that manifested for sheep and cattle large-intestine respectively.

Energy rather than nitrogen appears to be the limiting factor in hindgut fermentation (Orskov et al. 1970; Mason and Palmer 1973; Mason et al. 1977), the infusion of starch into the caecum of sheep resulting in significantly greater bacterial fermentation. Unlike the rumen very little soluble carbohydrate appears to reach the hindgut (McBee 1970; Keys 1974) with bacteria depending on the more fibrous components of the feed for their energy source. While increasing dietary fiber results in greater quantities of cellulose and hemicellulose reaching the large intestine bacterial fermentation may still be inhibited by the absence of a readily fermentable carbohydrate source which appears to be required for optimum bacterial growth (Hungate 1966).

The efficiency with which dietary VFA are utilised by swine (Bowland et al. 1971) suggests that the end products of hindgut microbial fermentation could provide a readily available energy source to the animal. The nutritional contribution of VFA to swine has been variously estimated as 5-28% of the animals maintenance energy requirement (Friend et al. 1964; Farrell and Johnson 1970; Imoto and Namioka 1978a; Kim et al. 1978b). Using barley based diets supplemented with maize or potato starch (Mason 1979; Mason and Just 1976) 18 and 44% respectively of the pigs maintenance energy was calculated as being derived from organic acids produced in the large intestine. Kass et al. (1980a; 1980b), using alfalfa as a fiber source, calculated that VFA production in the large intestine can contribute 4.8, 11.4, 14.0 and 12.0 % of the maintenance energy requirements of 89 kg pigs at 0, 20, 40 and 60 % alfalfa respectively.

In cattle and sheep reliable estimates of VFA production rates have become available through the use of isotope dilution techniques (Leng and Brett 1966). These techniques have not been used in swine, estimates of VFA production rates being based on in vitro techniques (Farrell and Johnson 1970; Imoto and Namioka 1978a) or arterial-venous differences (Friend et al. 1964, Imoto and Namioka 1978b). Calculations based on arterial-venous differences should be treated with considerable caution due to extensive metabolism of VFA in their passage through the

intestinal mucosa (Freeman et al. 1970; Stevens 1970; Imoto and Namioka 1978b).

The objectives of the present studies were to measure VFA concentrations and production rates, in swine fed three levels of dietary fiber, using a mixed caecal infusate of (1-^{14}C) acetate, (1-^{14}C) propionate and (2,3-^{3}H) butyrate.

C. MATERIALS AND METHODS

Cannulae and Surgical Procedure

Four crossbred (Yorkshire x Lacombe) castrated male pigs were fitted with single 'T' piece caecal cannulae at 40-50 kg liveweight. The cannulae used in this study were as described by Sauer (1976). These cannulae are extremely flexible due to their polyvinyl chloride construction and therefore less likely to be accidently removed by the animal. Replacement of these cannulae is easily performed and unlike the replacement cannulae reported by Decuypere et al. (1977) problems with intestinal perforation during the replacement procedure have not been encountered.

The animals were denied access to feed and water for 48 and 24 h respectively before commencement of surgery. Thirty minutes prior to surgery pigs received an intramuscular injection of 0.25 mg acepromazine maleate (Atravet, Ayerst Laboratory, Montreal Canada). Following the induction of anaesthesia by an intravenous injection of 20 mg/kg sodium pentobarbital (Nembutal, Abbott Laboratories, Ltd., Montreal

Canada) it was maintained with halothane introduced through a face mask, with oxygen, at a concentration of 1-2%. The animal was placed in a dorso-ventral position with heart rate, respiration rate and rectal temperature being monitored and strict aseptic techniques maintained throughout surgery. The caecum was exteriorised through a 7-10 cm posterolateral incision (right side) 5-10 cm posterior to the last rib. The site selected for the cannula was in the proximal end of the caecum (approximately 5 cm distal to the ileocaecal valve) such that, when the cannula was exteriorised through the body wall, the caecum would lie in a vertical position when the pig was standing. The cannula was exteriorised through a stab wound in the abdominal wall 2-3 cm distal to the incision. A polyvinyl chloride washer and clamp placed over the barrel of the cannula held the cannula securely in place. The resultant close contact between the caecum and the body wall promoted rapid adhesion between the two so that within 10 days the cannula could be replaced without danger of contaminating the abdominal cavity with digesta. Pigs received an intramuscular injection of 6 ml penicillin (300,000 iu/ml) daily for 3 days post surgery. Recovery was rapid with all animals eating normally within 5 days.

Measurement of VFA Production Rates

Infusion technique. Radioisotopes (acetate, propionate and butyrate) were infused into the caecum through a Tygon

catheter (Norton plastics and synthetics division) (internal diameter 0.8 mm and external diamter 2.3 mm) using individual lambda pumps (model 1302, Harvard Instruments, 150 Dover Rd., Millis, Mass. USA) for each animal. Control over the site of infusion and uniform distribution of the infusate was obtained in the following manner. A 30 cm length of copper wire was inserted (35 cm) into the end of the catheter and the latter was heat sealed. A 22 gauge needle was heated over a bunsen burner and used to make 20-30 perforations around the barrel of the terminal (5-6 cm) end of the catheter. The presence of the copper wire caused a slight build up in pressure as the infusate was forced passed it with a resultant band spray effect as the liquid was ejected through the perforations. Sampling from the caecum showed that this method resulted in more uniform distributrion of infusate than was achieved with a simple catheter placed in the caecum. The catheter was inserted through the cannula and held in place by bending it sharply so that it ran parallel to the outer wall of the barrel of the cannula. In this position it could be taped securely in place allowing the rubber stopper of the cannula to be withdrawn without interfering with the position of the catheter.

<u>Site of infusion.</u> The effect of site of infusion on the uniformity of isotope distribution in the caecum was examined under both single injection and continous infusion conditions. Uniformity of distribution was checked by

withdrawing 5-10 samples (5-10 ml) from various sites in the caecum using a (internal diameter 5 mm) polyethylene tube attached to a 50 cc syringe, and measuring radioactivity. In general the uniformity of distribution improved as the site of infusion was progressively moved towards the posterior aspect of the caecum. Optimum conditions were achieved when the infusion site was in the ventral 5-6 cm of the caecum. Under such conditions the coefficient of variation, of isotope concentration, among 10 replicate samples taken from various sites in the caecum, was less than 7%. Consequently this procedure was employed throughout the experimental period.

Sampling method. Because of the viscous nature of the caecal contents difficulties were experienced in obtaining samples in excess of 5 ml. The use of a vacuum pump also proved unsuccessful. As the homogeneity of isotope distribution in the caecum was good the following procedure was found to give representative samples and allowed sampling to be done without disturbance to the animal. The rubber stopper on the cannula was removed and a weighed 20 ml scintillation vial held over the mouth of the cannula was rapidly filled due to the backpressure associated with caecal contractions. The pH of the sample was immediately determined by inserting a combination electrode (Fisher Scientific Co.) directly into the vial. The sample was weighed, quick-frozen with liquid nitrogen and stored at -20° until analyzed. Total fecal samples were collected as voided, weighed and mixed. Two

subsamples (approx. 20g) from each collection were placed in scintillation vials, quick frozen with liquid nitrogen and stored at -20° c.

Infusion and sample collection. In experiment 1, 2 and 3 the continuous infusion lasted for 8-12 h at a rate of 0.16-0.21 ml/min. This infusion rate supplied approximately 0.48 microcuries/min (1-^{14}C) acetate, 0.21 microcuries/min (1-^{14}C) propionate and 1.16 microcuries/min (2-3-^{3}H) butyrate. Caecal samples were collected immediately prior to commencement of continuous infusion and each hour thereafter for 24 h.

Experiment 1

After surgery the four cannulated pigs were placed in metabolism crates in a room maintained at 20-22 °C. All four pigs received 2.74 kg daily of diet 1 (Table 1), in pelleted form, in 24 equal feeds from an automatic feeding device (Turner et al. 1980).

Pigs were allowed 18 days to recover from surgery and adjust to the feeding regime prior to the commencement of a 10 d total fecal collection. Daily total fecal collections from each animal were dried in a forced air oven at 60°C for 3 days. Dried daily samples from each animal were ground through a 0.84 mm screen, mixed and subsampled for subsequent analysis.

At the conclusion of the digestibility study VFA production rates were measured as described previously.

Experiment 2

Ten days after completion of experiment 1 the pigs were fed the 3 diets shown in Table 1. The experimental design was incomplete block with 4 pigs and 3 diets in each of 3 periods. Pigs were fed 800 g at 0500, 1300 and 2100 h daily. Volatile fatty acid production rates were determined, as described previously, in each period, after a 7-day adaptation period to the diet.

Experiment 3

At the conclusion of experiment 2 the pigs were moved to individual pens (concrete floored without bedding) and fed diet 3 (ad libitum) for 13 consecutive weeks. The animals were then returned to the metabolism crates and allowed 2 weeks to adjust to their environment and dietary regime. During this period and throughout experiment 3 pigs were fed 2.4 kg daily of diet 3 in 3 equal feeds at 0600, 1400 and 2200 h.

Chemical Methods

VFA analysis.

Sample preparation - Plastic scintillation vials containing caecal and fecal samples were thawed and allowed to equilibrate overnight at room temperature. Following removal of screw caps the vials were centrifuged for 15 min. at 20,000 g. An Oxford pipette was used to withdraw the supernatant. The vials were then lyophilised and dry matter determined.

Total radioactivity - A 0.2 ml aliquot of supernatant was pipetted into a scintillation vial to which 15 ml Aquasol (New England Nuclear, Mass.) was added for determination of total sample radioactivity which was later used to calculate the percentage of total radioactivity associated with acetate, propionate and butyrate. Samples were counted for 20 min. in Searle Mark III (Tracor Northern Inc. Wi. USA, 53562) scintillation counter using dual label (^{14}C and ^{3}H) counting program with variable window settings to optimise counting efficiency while minimizing spillover.

VFA separation and concentrations- Two ml of supernatant from centrifuged caecal and fecal samples was placed in Bio-Gamma vials (Beckman Instruments) to which was added 0.2 ml of a 85% phosphoric acid solution in which 3.44 mg iso-valeric acid was present as an internal standard. Duplicate samples were injected into a gas-liquid chromatograph (Bendix model 2526-1) with a column (1.24 m x 5 mm) packed with Chromsorb 101 (80-100 mesh). The column was fitted with a splitter which directed 15% to the flame ionization detector and the remaining 85% to a 7 cc glass trap, packed with cotton wool soaked in ethanolamine. By changing traps between peaks separate collection of acetate, propionate and butyrate peaks was possible. The cotton wool was placed directly in a scintillation vial and quantitative recovery achieved by rinsing the trap with approximately 1 ml methanol which was shown to give 100% recovery of radioactivity in the trap. Following the addition of 15 ml

Aquasol samples were counted for 20 min in Mark III scintillation counter using the dual label program. The use of cotton wool, methanol or ethanolamine did not contribute to sample quenching. The response for individual VFA and their molar concentrations were calculated from the integrator response of the flame ionization detector by reference to the internal standard and a standard solution (44.15 mmoles acetate, 8.67 mmoles propionate, 4.84 mmoles buytrate and 4.11 mmoles iso-valeric) injected between every 10 samples.

Proximate analysis- Proximate analysis of feed and fecal samples were performed by the standard methods of the Association of Official Agricultural Chemists (1975).

Statistical analysis

All statistical analyses were conducted on an individual pig basis. Data were analyzed using least squares analysis of variance for unequal numbers (Harvey 1960) adjusting for all identified sources of variation . Means for significant treatment differences were compared using Student - Newman - Keuls multiple range test (Steel and Torrie 1960) with adjustments for unequal number of observations per mean.

D. RESULTS

Effect of Cannulation

At nine months post surgery two of the above animals were slaughtered and post-mortem examinations conducted. While the distal 0.1 to 1.1 m of the ileum was mildly dilated no evidence of any smooth muscle reaction or mucosal abnormality was evident. In general the examination revealed that the caecum was well adhered to the body wall at the point of entry of the cannula. While no significant morphological alteration was observed in the intestinal tract above normal counts of coliforms were found in all sections of the small intestine with similar counts occurring in the duodenum, jejunum and ileum.

Pool Size

The weight of caecal contents has been variously determined as 82-421 g (Kim et al. 1978a; 1978b) and 195-694 g (Friend et al. 1963a). Among the factors which appear to influence the weight of caecal contents are the breed of the animal and the experimental diet. The pig caecum is generally considered to have a volume of only a few hundred ml (McBee 1977). However, estimates of pool size obtained in the present study using single caecal infusions of isotopes were always greater than 450 ml and as high as 920 ml. These results would appear to indicate that the VFA pool being measured in the present study included both the caecum and proximal colon. The pattern of caecal contractions, measured using a pressure transducer inserted in the caecum, appear

to confirm that free reversible movement of digesta occurs between the caecum and proximal colon. All estimates of VFA production rates therefore refer to the caecum and proximal colon.

Experiment 1

One pig was withdrawn from this experiment as steady state tracer levels were not observed, in caecal samples, after a 9 h continuous isotope infusion.

Dry matter (DM) intake was equal for all animals (Table 2) but considerable animal variation was observed in DM, nitrogen and crude fiber (CF) digestibility.

Significant differences among animals were observed in VFA concentration, VFA molar percent and VFA ratios (Table 3). The mean VFA concentrations in caecal fluid were, respectively, 79.1, 33.0, 9.9 and 122.0 mmoles/l for acetate propionate, butyrate and total (acetate+ propionate + butyrate) VFA. Average caecal pH over the 24 h sampling period was 6.17.

Gross VFA production rates for each animal are presented in Table 4. Production rates (mmoles/h) for acetate, propionate and butyrate respectively varied between pigs from 45.0 to 58.4, 12.8 to 16.0 and 4.3 to 7.0. When corrected for interconversions between acetate and butyrate the average net production rates were, for acetate propionate and butyrate respectively, 42.57, 14.33 and 4.86 mmoles/h. Interconversions were calculated as described by Leng and Leonard (1965) and Leng and Brett (1966). As these

authors show a very low rate of interconversion between propionate and both acetate and butyrate no attempt has been made to correct for their interconversions.

The gross energy values for acetate, propionate and butyrate have been calculated (Leng and Leonard 1965) as 14.6 kJ/g, 20.7 kJ/g and 24.9 kJ/g, respectively. Thus assuming complete absorption of VFA the energy supplied by acetate, propionate and butyrate ranged between 1491 and 1947 kJ/d (Table 4). The maintenance energy requirement of 50-60 kg pigs is about 8550 kJ/d digestible energy (ARC 1967). The contribution of VFA to the animals maintenance energy requirements would therefore range from 17.4 to 22.8%.

Experiment 2

Mean caecal VFA concentrations, molar percent and ratios for experiment 2 are presented in Table 5. The average total VFA concentrations for pigs fed diet 1 in experiment 2 was 122.7 mmoles/l in contrast to 122.0 mmoles/l for pigs fed the same diet in experiment 1.

The addition of 27.3 and 52.0% alfalfa to diets 2 and 3 respectively resulted in significantly higher acetate concentration than observed with diet 1. Acetate concentration in the caecum tended to be lower in pigs fed the 15 % crude fiber diet than for those fed diet 2. Increasing dietary fiber significantly lowered propionic acid concentration. Consequently total VFA concentrations were significantly lower in pigs fed diet 3 than those fed

diet 2. Molar percent acetic acid significantly increased with increasing fiber level in the diet while that of propionic decreased. In general increased dietary fiber resulted in significantly higher acetate/propionate ratio. Molar percent for butyrate for pigs fed diets 2 and 3 did not differ significantly but both were significantly lower than that calculated for pigs fed diet 1.

The influence of animal variation was particularily evident in propionate concentrations (Table 6) with caecal concentrations ranging between 22.1 and 35.3 mmoles/l. These differences in turn were reflected in significant differences between animals in total VFA concentrations, VFA molar percent and ratios.

The largest production rates for acetate, propionate and butyrate were observed for pigs fed the 10% CF diet (Table 7). While VFA production rates were lower when pigs were fed 15% CF diets they were still 13.1% higher than determined for pigs fed the low fiber control diet. When adjusted for interconversions between acetate and butyrate, total VFA production rates were calculated to account for respectively, 867, 1294 and 946 kJ/d for 5, 10 and 15% CF diets. Assuming complete absorption of VFA this represents an energy contribution of 10.1, 15.1 and 11.1% of the animals maintenance energy requirement for pigs fed 0, 27.3 and 52.0% alfalfa respectively.

<u>Experiment 3</u>

As in experiments 1 and 2 considerable animal variation

was again observed. While no significant differences between animals were observed in acetate concentration, significant differences were observed in propionic and butyric acid concentrations and hence VFA molar percent and ratios. Volatile fatty acid concentrations were much lower in this experiment (Table 8) than observed for pigs fed a similar diet in experiment 2 (81.8 vs 115.3 mmoles/l). However, total production rates of VFA were similar (58.2 vs 56.9 g/d) in both experiments. Again assuming complete absorption of VFA a production rate equivalent to 999 kJ/d would supply 9.5% of the maintenance energy of the animal.

At the conclusion of these experiments VFA production rates were estimated using a single isotope injection rather than a continuous infusion system. The single injection method resulted in considerable variation in estimates of pool size. The resulting variability in production rates of VFA were considered excessive and therefore will not be presented. However VFA concentrations (Table 9) show a similar pattern to that observed in the previous studies. The highest and lowest concentrations respectively were observed for pigs fed the 10 and 4.8% CF diets. The total VFA concentrations for the 3 diets were considerably lower than observed 15 weeks earlier in experiment 2. Enhanced absorption or a shift in the site of fermentation, associated with the physiological age of the animal or its 15 week adaptation to the 15% CF diet may have contributed to these differences. As in the previous studies significant

animal variation occured in VFA concentrations and ratios.

E. DISCUSSION

The present experiments appears to confirm the report of MacRae and Wilson (1978) that the use of 'T'-piece cannulae do not result in gross morphological changes in the intestine.

VFA concentrations and ratios

Caecal VFA concentrations were highest in experiment 1 where pigs were fed in 24 equal feeds per day. However the level of feed intake was also higher than experiment 2 and this may have resulted in larger quantities of carbohydrate reaching the caecum. Total caecal VFA concentrations reported here (80.9-130.4 mmoles/l) are considerably higher than the 23.7 mmoles/l observed by Kass et al. (1980b). Friend et al. (1963a) observed total VFA concentrations of 176-246 mmoles/l. These levels were for pigs fed diets with a high lactose content where lactic acid contributed up to 27% of the total organic acid concentration. The values reported by Imoto and Namioka (1978a) (approximately 125 mmoles/l) are in agreement with the results of the present study. The low VFA values reported by Kass et al. (1980b) are particularily surprising in view of the fact that the diets used (Alfalfa as fiber source) were somewhat similar to those fed in the present investigation. While some of the differences in caecal VFA concentrations are undoubtedly due to animal and dietary variation, it is likely that the

experimental techniques used in measuring VFA concentrations will strongly influence the results obtained. Previous estimates of VFA concentrations have for the most part been based on measurements made in sections of the intestinal tract following slaughter of the animal. Post mortem changes, therefore, may have exerted a considerable influence on the results obtained.

The average caecal molar percent of acetate, propionate and butyrate in experiment 1 were respectively 64.8, 27.1 and 8.1. While these results are in accord with those reported by Kass et al. (1980b) they differ from those recorded by Friend et al. (1963a; 1963b). In pigs fed a diet similar to that used in the present study (Friend et al. 1963a) the percentage proportions of acetate, propionate , butyrate and lactate respectively, were 47.2, 31.2, 5.0 and 16.4. However in the same study pigs fed a diet high in bran were found to have proportions of 60, 32 and 7.5% for acetate, propionate and butyrate, respectively.

As the level of alfalfa in the diet was increased from 0 to 27.3% caecal concentration of acetate increased (Table 5 and 9) while that of propionate decreased. These results are in agreement with those of Friend et al. (1963a) and Kass et al. (1980b). However when the level of alfalfa was increased to 52% total VFA concentrations declined while the acetate/propionate ratios continued to increase. Caecal VFA concentrations for pigs fed diet 1 in experiments 3 (Table 8) were considerably lower than observed in experiment 1.

The age of the animal or the 15 week adaptation period to the high fiber diet appeared to have a depressing effect on caecal VFA concentrations. However these results could also be explained by enhanced absorption or a shift in fermentation towards the distal colon.

VFA production rates

Production rates of VFA were highest for pigs fed diet 1 in experiment 1 (61.8 mmoles/h) and were least for pigs fed diet 1 in experiment 2 (32.46 mmoles/h). These differences were undoubtedly due in part to the higher feed intake of pigs in experiment 1. However, the frequency of feeding (hourly versus 3 times daily) may also have influenced the results obtained.

Total caecal VFA production rates in experiment 2 were significantly higher in pigs fed 27.3% alfalfa than those fed 0% alfalfa. However, the addition of 52% alfalfa resulted in intermediate values for VFA production. It appears that, where alfalfa is used as a source of fiber, VFA production increases up to about 10% CF in the diet. Where the fiber level of the diet is increased to 15% VFA production tends to decrease slightly. Increased dietary fiber results in greater quantities of fibrous material entering the hindgut. However, this material is likely to be less readily available for microbial fermentation than starch or soluble carbohydrate. In consequence fermentation may tend to occur at a later stage in the large intestine. While VFA production rates in the caecum or proximal colon

may tend to decline on a very high fiber diet, this may be compensated for by increased production in the distal colon. Caecal VFA production rates of 7.23 to 18.9 mmoles/h have been reported by Kim et al. (1978a) while total production rates for the caecum and colon of 23.6 to 52.1 mmoles/h have been calculated by Imoto and Namioka (1978a). The contribution of caecal VFA to total VFA production has been estimated as 15.8 to 19.0% (Imoto and Namioka 1978a) and as high as 29.9% (Kim et al. 1978a; 1978b).

A 15 week adaptation period to the 52% alfalfa diet appeared to have little influence on VFA production rates. Pigs fed the 15% crude fiber diet for 15 weeks had similar VFA production rates (Table 8) to those observed following a 2 wk adaptation period to the diet (Table 7). These results would appear to confirm the report of Cunningham et al. (1962) who found no evidence of increased ability of swine to digest cellulose following a 15 wk adaptation period. However the type of fiber and age of the animal may influence the results obtained. Moreover, as VFA production has not been measured in the total hindgut a shift in fermentation towards the terminal colon could confound the results.

It is clear from the present series of experiments that substantial VFA production occurs in the large intestine of swine regardless of the level of fiber in the diet. Microbial fermentation, therefore, makes an important contribution to the utilization of dietary energy by swine.

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Table 1. Formulation, composition and proximate analyses of experimental diets

Diet No.	1	2	3
Ingredient (%)			
Barley	83.5	61.9	42.6
Soybean meal	12.5	7.3	2.4
Dehydrated alfalfa	-	27.3	52.0
Iodized salt	0.5	0.5	0.5
Calcium phosphate	1.5	1.5	1.5
Calcium carbonate	1.0	0.5	-
Vitamin-mineral premix+	1.0	1.0	1.0
	100.0	100.0	100.0
Analysis			
a) Calculated (% as fed)			
Calcium	0.73	0.91	1.0
Phosphorus	0.66	0.61	0.67
Lysine	0.7	0.72	0.72
Methionine & cystine	0.56	0.53	0.51
Digestible energy (MJ/kg)	12.9	10.9	9.1
b) Determined (% as-fed)			
Nitrogen (%)	2.32	2.43	2.45
Crude fibre (%)	4.78	9.85	15.01

\+ Contribute the following per kg of diet: Zn, 120 mg; Cu, 10 mg; Mn, 48 mg; Fe, 100 mg; Se, 0.1 mg; Vitamin A, 7500 IU; Vitamin D, 700 IU; Vitamin E, 45 IU; Riboflavin, 12 mg; Niacin, 40 mg; Calcium pantothenate, 27 mg; Vitamin B_{12}, 28 μg

Table 2. Daily dry matter (DM) nitrogen and crude fiber (CF) intake excretion and digestibility in experiment 1.

Diet No.	1	1	1	
Pig #	53	56	130	SEM
DM intake (g/day)	2462.	2462.	2462.	10.5
DM excretion (g/day)	604.4	547.3	553.1	21.4
DM digestibility (%)	75.5	77.8	77.5	1.3
Nitrogen intake (g/day)	63.5	63.5	63.5	0.2
Nitrogen excretion (g/day)	16.6	15.1	15.3	0.6
Nitrogen digestibility (%)	73.8	76.2	75.9	1.2
CF intake (g/day)	130.5	130.5	130.5	0.5
CF digestibility	24.4	31.7	30.9	0.8

Table 3. Volatile fatty acid (VFA) concentrations+, molar percent VFA ratios and caecal pH for pigs fed diet 1 in experiment 1.

Pig #	53	56	130	Mean
VFA concentrations (m molar)				
Acetic (A)	90.0a‡±3.2	76.8b±4.0	70.3b±2.7	79.1±1.9
Propionic (P)	37.1a±1.5	30.5b±1.8	31.5b±1.2	33.0±0.9
Butyric (B)	10.6a±0.7	11.1ab±0.9	8.0c±0.6	9.9±0.4
Total (A+P+B)	137.7a±4.8	118.4b±6.0	109.8b±4.0	122.0±2.9
VFA molar percent				
Acetic	65.4 ±0.9	64.9 ±1.1	64.0 ±0.7	64.8 ±0.5
Propionic	26.9a±0.7	25.7a±0.8	28.8b±0.5	27.1 ±0.4
Butyric	7.7a±0.4	9.4b±0.5	7.2a±0.3	8.1 ±0.2
VFA ratios				
A/P	2.44ab±0.09	2.55a±0.11	2.26b±0.07	2.4 ±0.05
A/B	8.73 ± 0.51	7.29 ±0.63	9.10 ±0.42	8.4 ±0.3
A/(P+B)	1.90 ± 0.07	1.88 ±0.09	1.80 ±0.06	1.9 ±0.04
Caecal pH	6.10 ± 0.20	6.25 ± 0.25	6.16 ±0.26	6.17±0.20

a,b,c means in the same row with different letters are different (P<0.05)

\+ determined in caecal fluid

‡ each value is the mean of 24 observations taken over a 24h period

Table 4. Volatile fatty acid production rates+ of pigs fed diet 1 in experiment 1.

Pig #	53	56	130	Mean	SEM
Gross production rate (mmoles/h)					
Acetate (A)	45.0	58.4	46.3	49.9	8.2
Propionate (P)	12.8	16.0	14.2	14.3	2.1
Butyrate (B)	4.3	7.0	4.9	5.4	0.9
TOTAL (A+P+B)	62.1	81.4	65.4	69.6	9.7
Interconversions					
Acetate to butyrate					
percent	71.5	69.7	61.9	67.7	25.6
mmoles/h	6.15	9.76	6.07	7.33	2.6
Butyrate to acetate					
percent	2.1	2.4	2.0	2.17	0.2
mmoles/h	0.47	0.70	0.46	0.54	0.1
Net production rates					
Acetate					
mmoles/h	38.85	48.64	40.23	42.56	7.1
g/d	55.94	70.04	57.93	61.30	10.3
kJ/d	819	1026	849	898	151.8
Propionate					
mmoles/h	12.8	16.0	14.2	14.3	2.1
g/d	22.7	28.4	25.2	25.4	3.7
kJ/d	471	590	524	528	79.2
Butyrate					
mmoles/h	3.83	6.30	4.44	4.86	0.9
g/d	8.1	13.31	9.38	10.26	1.8
kJ/d	201	331	233	255	45.9
Total (A+P+B)					
mmoles/h	55.48	70.94	58.87	61.72	7.3
g/d	86.74	111.75	92.51	96.96	11.5
kJ/d	1491	1947	1606	1681	173.6

\+ see text for details

Table 5. Average caecal volatile fatty acid (VFA) concentrations[+], molar percent and ratios for pigs fed 3 levels of fiber in experiment 2.

Crude Fibre %	5	10	15	
Diet No.	1	2	3	SEM
VFA concentrations (mmolar)				
Acetic (A)	78.0a[‡]	87.3b	84.8b	1.60
Propionic (P)	34.0a	28.8b	22.5c	0.74
Butyric (B)	10.7a	9.5ab	8.0b	0.62
Total (A+P+B)	122.7ab	125.6a	115.3b	2.44
VFA molar percent				
Acetic	64.2a	69.6b	74.2c	0.38
Propionic	27.4a	22.8b	19.0c	0.28
Butyric	8.4a	7.6b	6.8b	0.28
VFA ratios				
A/P	2.41a	3.06b	4.25c	0.07
A/B	8.49a	9.67b	11.93c	0.27
A/(P+B)	1.85a	2.30b	3.12c	0.05

\+ determined in caecal fluid

‡ each value is the mean of 96 observations

a-c means in the same row with different letters are different ($P<0.05$)

Table 6. Average caecal volatile fatty acid (VFA) concentrations+, molar percent and ratios for individual animals fed 3 diets in experiment 2.

Pig #	53	56	61	130	SEM
VFA concentrations (mmolar)					
Acetic (A)	80.1‡	83.6	84.4	85.4	1.80
Propionic (P)	22.1a	27.2b	29.2b	35.3c	0.83
Buytric (B)	7.3	8.6	12.1	9.6	0.70
Total (A+P+B)	109.5a	119.4b	125.6bc	130.4cd	2.76
VFA molar percent					
Acetic	73.6a	70.4b	67.7c	65.5d	0.43
Propionic	19.9a	22.3b	23.1bc	27.0d	0.32
Butyric	6.5a	7.2ac	9.1b	7.4c	0.31
VFA ratios					
A/P	4.07a	3.38b	3.06c	2.46d	0.08
A/B	12.35a	10.47b	8.04c	9.28d	0.31
A/(P+B)	3.04a	2.52b	2.19c	1.93d	0.06

+ determined in caecal fluid

‡ each value is the mean of 72 observations taken over three 24 h periods.

a-d means in the same row with different letters are different ($P<0.05$)

Table 7. Average volatile fatty (VFA) production rates in experiment 2.

Diet	1	2	3
<u>Gross production rate (mmoles/h)+</u>			
Acetate (A)	25.85 ± 5.2	40.98 ± 6.1	31.86 ± 5.2
Propionate (P)	8.38 ± 1.1	11.20 ± 1.3	7.10 ± 1.1
Butyrate (B)	1.95 ± 0.64	2.40 ± 0.72	1.96 ± 0.64
TOTAL (A+P+B)	36.18 ± 6.3	54.58 ± 7.6	40.92 ± 6.8
<u>Net Production rate (mmoles/h)+</u>			
Acetate			
mmoles/h	22.50 ± 4.5	37.28 ± 5.5	28.29 ± 4.6
g/d	32.40 ± 7.2	53.68 ± 7.9	40.74 ± 6.7
kJ/d	475.00 ± 104.	786.00 ± 115.	597.00 ± 98
Propionate			
mmoles/h	8.38 ± 1.1	11.20 ± 1.3	7.10 ± 1.1
g/d	14.90 ± 2.0	19.82 ± 2.3	12.57 ± 2.0
kJ/d	309.00 ± 40.	411.00 ± 48.	261.00 ± 40.
Butyrate			
mmoles/h	1.58 ± 0.52	1.83 ± 0.55	1.69 ± 0.55
g/d	3.33 ± 1.1	3.86 ± 1.17	3.57 ± 1.17
kJ/d	83.00 ± 28.	96.00 ± 29.	89.00 ± 29.
Total (A+P+B)			
mmoles/h	32.46a	50.31b	37.08ab
g/d	50.63a	77.36b	56.88ab
kJ/d	867a	1294b	946ab

+ see text for details

Table 8. Average caecal volatile fatty acid (VFA) concentrations*, molar percent, ratios and production rates in experiment 3.

	Acetic	Propionic	Butyric	Total
Concentration (mmoles)	57.9 ± 1.4+	17.7 ± 0.5	6.2 ± 0.2	81.8 ± 2.0
Molar %	70.9 ± 0.2	21.6 ± 0.2	7.5 ± 0.1	100.0 ± 0.3
Gross production rate‡				
mmoles/h	32.18 ± 5.9	6.39 ± 1.0	3.73 ± 0.8	42.3 ± 7.7
Net production rate‡				
mmoles/h	27.34 ± 5.0	6.39 ± 1.0	3.54 ± 0.8	37.27± 5.9
g/d	39.37 ± 7.2	11.35 ± 1.8	7.48 ± 1.6	58.2 ± 10.6
kJ/d	577.0±106.1	236.0±37.8	186.0±40.2	999.0± 183

* determined in caecal fluid

\+ each value is the mean of 24 observations taken over a 24h period

‡ see text for details

Table 9. Average caecal volatile fatty acid (VFA) concentrations[+], molar percent and ratios for pigs fed 3 levels of fibre after a 15 week adaptation to a 15% crude fiber diet.

Crude fib %	5	10	15	
Diet No.	1	2	3	SEM
VFA concentrations (m molar)				
Acetic (A)	55.7a	63.9b	59.8ab	2.4
Propionic (P)	30.1a	25.3b	18.4c	1.6
Butyric (B)	9.0a	7.5ab	6.2b	0.6
Total (A+P+B)	94.8	96.7	84.4	4.1
VFA molar percent				
Acetic	59.5a	66.4b	70.6c	1.0
Propionic	31.4a	25.9b	22.0c	0.81
Butyric	9.1a	7.7b	7.4b	0.35
VFA ratios				
A/P	2.02a	2.81b	3.27c	0.10
A/B	7.04a	9.41b	9.94b	0.46
A/(P+B)	1.54a	2.15b	2.45c	0.08

+ determined in caecal fluid

VI. GENERAL SUMMARY

The need for caution when attributing a cause and effect relationship between level of fiber and animal performance is illustrated by reference to the present rapeseed meal (RSM) study. The association of inhibitory substances with the fiber source makes it extrmely difficult to isolate the influence of fiber per se. While the high level of fiber in RSM has frequently been implicated in reduced animal performance, low fiber varieties of RSM or dehulled RSM used in the study reported here were not associated with improved animal performance over that obtained with the high fiber variety of RSM.

It is clear from the present results that the disagreement in the literature on the influence of fiber on performance, carcass composition and digestibility coefficients is due, at least in part, to qualitative and quantitative differences associated with changes in the protein and energy level of the diet. When comparisons are being made between various studies consideration must also be given to factors other than the level and source of fiber in the diet. Changes occurring in energy and protein as a result of the mode of addition of the fiber cannot be ignored. Pigs possess the ability to increase feed intake to compensate for the digestible energy dilution upon addition of fiber. However this ability appears to be related to the length of time to which the animal has had to adapt to the diet. In the experiments reported here pigs receiving 10% CF

diets from 22 kg to slaughter at 92 kg had similar backfat to control animals fed 4% CF diet.

It is generally assumed that increasing dietary fiber results in depressed nitrogen digestibility. However following a 10 wk adaptation period to the fiber source the level of fiber did not influence nitrogen digestibility. Where depressed nitrogen digestibility is observed it is more likely to be due to increased bacterial nitrogen assimilation in the hindgut rather than a reduction in the true nitrogen digestibility of the diet. A possible exception is where the fiber source contributes appreciable quantities of nitrogen which has lower availability. Currently available methods for measuring the chemical components of dietary fiber are inadequate. The composition of dietary fiber cannot be measured with the precision which is currently available for other dietary constituents. The use of crude fiber (CF) remains popular because of the absence of a reliable alternative method which gives similar precision and ease of operation. A chemical and nutritional evaluation of currently available methods for fiber analysis is long overdue. The challenge for the chemist is the development of a method which separates the various chemical components of dietary fiber while still being amenable to routine feed evaluation.

The results of the isotope dilution study (chap v) indicate that extensive microbial fermentation occurs in the hindgut of swine. Total caecal VFA concentrations

(80.9-125.6 mmoles/l) varied with the feeding regime and experimental diet. The length of adaptation (2 vs 15 wks) to the fiber source had no significant influence on VFA production rates. The production rates of VFA increased as the crude fiber level of the diet was raised to 10%. However when diets with 15% crude fiber were fed VFA production rates tended to decline below those observed for the 10% CF diet. The contribution of VFA to the pigs maintenance energy requirement (9.5-22.8%) verifies the important role of microbial fermentation in the utilization of dietary energy by swine.

VII. APPENDIX I. DETERMINATION OF DYSPROSIUM CERIUM AND CHROMIUM BY NEUTRON ACTIVATION ANALYSIS

A. ABSTRACT

An instrumental neutron activation analysis (INAA) procedure has been developed for the measurement of dysprosium (Dy), cerium (Ce) and chromium (Cr) using the Canadian SLOWPOKE nuclear reactor. These elements were included in animal feed at 25 ppm, 61 ppm and 0.34% respectively. Concentrations in feed and digesta samples were determined by measuring, with a Ge(Li) detector coupled to a 4096 multichannel analyser, the radioactivity induced by neutron irradiation. Quantitative analysis procedures based on ^{165m}Dy (half-life : $T_{\frac{1}{2}}$=1.26 min) ^{51}Cr ($T_{\frac{1}{2}}$=28 d) and ^{141}Ce ($T_{\frac{1}{2}}$=32.6 d) were developed after a study of the major contributions to induced background activity in both feed and fecal materials. For short irradiations, ^{28}Al is the dominant induced background activity; for longer irradiations (> 2 h), ^{38}Cl, ^{56}Mn and ^{24}Na dominate. Inert markers with radioisotopes of halflives less than several minutes (^{165m}Dy) or longer than several days (^{51}Cr and ^{141}Ce) are most amenable to instrumental analysis. The application of the methods developed to routine analysis is discussed from the viewpoint of sample throughput and analysis time.

B. INTRODUCTION

While radioisotopes are gaining in popularity as markers in studies of digestive physiology, the difficulties associated with animal contamination and the disposal of large quantities of fecal waste remain. These problems can be avoided by the inclusion of a nonradioactive marker in the feed with subsequent quantitative analysis of its concentration in feed and digesta material. Generally this incorporation must be at the trace level to avoid adverse physiological effects (Hutcheson et al. 1975). Wet chemical analysis or instrumental techniques such as mass spectrometry, x-ray fluorescence and atomic absorption can be used but generally involve tedious procedures. If the markers are suitably chosen, instrumental neutron activation analysis (INAA) can provide a degree of sensitivity not often available with other methods. In addition, both the nondestructive nature of the analysis and the ease with which the trace levels of single or multiple markers (Luckey et al. 1975; 1977; 1979a) can be quantitatively measured, in a single determination, in widely different biological matrices (Clemente et al. 1977; Ellis et al. 1977) without chemical work-up greatly encourages the development of INAA procedures for use by animal scientists.

The applicability of inert markers coupled with neutron activation analysis has been the subject of a limited number of studies (Ellis 1968; Olbrich et al. 1971; Young 1975; Martz 1971). These studies have been limited to ruminant

animals and with the exception of Ellis (1968) longer lived radionuclides have been used despite the decreased analysis time potentially available with the shorter lived nuclides.

The objectives of this study were to develop an analytical procedure for the INAA of digestive markers using the Canadian SLOWPOKE nuclear reactor and to devise an optimum procedure for routine analysis which would readily lend itself to automation.

Of the various potential inert markers reported in recent literature (Gray and Vogt 1974; Luckey et al. 1979b) dysprosium (Dy), cerium (Ce) and chromium (Cr) were chosen for this study. Both Ce (Huston and Ellis 1968; Olbrich et al. 1971) and Dy (Ellis 1968; Young et al. 1975) have been shown to possess many of the properties of ideal inert markers and are, in addition, good candidates for INAA at trace levels. Dissatisfaction expressed with the analysis of chromic oxide by colorimetric methods (Carew 1973) prompted this investigation into the possibility of its measurement by INAA. It was therefore decided to develop an analytical procedure for these three elements although other markers are possible and have been proposed (Gray and Vogt 1974).

A survey of the trace elements observable by INAA in both the feed and fecal material of swine was carried out to ascertain whether any were suitable candidates for use as internal markers or could interfere with added marker measurements. This information about the sample background activity used in designing the INAA procedures for Dy, Ce

and Cr should prove a valuable aid in in both the selection of other markers and in determining the feasibility of using this INAA scheme for mineral metabolism studies (Budinger et al. 1972; Kostic et al. 1977; Schelenz 1977; Tanner and Friedman 1977).

C. MATERIALS AND METHODS

Animals and Diets

Four pigs of average initial weight 67Kg were confined to stainless steel metabolism crates and fed a diet (Table 1) formulated to meet or exceed NRC nutrient requirements. The animals were fed twice daily: 0.7Kg at 0800 h and 1.4Kg at 1600 h.

Dysprosium (25 ppm elemental Dy), Cr (0.34% elemental Cr) and Ce (61 ppm elemental Ce) were added to the feed as $DyCl_3 6H_2O$,Cr_2O_3 and $(NH_4)_4Ce(SO_4)_4 2H_2O$ respectively. Because addition of these markers is at the ppm level care was taken to ensure homogeneity. Dysprosium, Ce and Cr were mixed dry with a similar weight of finely ground feed. More feed was added gradually until complete mixing at the appropriate concentration was achieved. Random grab samples of the feed showed that departures from homogeneity for Dy and Ce were less than 1 ppm.

Sample Collection and Preparation

Following an adaptation period of 7 days, total fecal

output was collected daily from each animal for 7 consecutive days. The daily total collection for each pig was mixed wet and three 50 g random subsamples were taken for analysis.

Samples (100 mg) of feed and freeze-dried fecal matter were accurately weighed into acid cleaned 1.5 cm^3 polythelene vials which were in turn enclosed in the 7 cm^3 polyethelene capsules used as irradiation rabbits. During all sample preparation stages, clean conditions were maintained. Prior to counting, the sample vials were removed from the protective outer containers, thereby reducing background counts due to any external contamination.

Nuclear Reactor and Gamma Spectroscopy System

All neutron irradiations and gamma ray analyses were conducted at the University of Alberta SLOWPOKE reactor. SLOWPOKE is an acronym for the Canadian pool reactor developed and marketed by Atomic Energy of Canada Ltd. and is derived from Safe LOW POwer Kritical Experiment. As there are a number of equivalent and independent irradiation sites in the SLOWPOKE reactor, the INAA of digestive samples could be carried out at the same time as other irradiations in the reactor. Due to the reactor's inherent flux stability and reproducibility (Kay et al. 1973, Jervis et al. 1977) the constant inclusion of elemental standards, flux monitors and corrections for reactor geometry (Young et al. 1975) are not required, thereby greatly simplifying the analytical

procedure. A pneumatic rabbit system, with a transit time (in or out of the reactor) of approximately 1.5 s. and an automatic irradiation time controller, made the analysis of shortlived radionuclides possible.

The gamma ray spectroscopy system consisted of a solid state Ge(Li) coaxial detector (18.5% relative efficiency and 1.93 kev FWHM at 1332 kev) coupled to a 4096 channel ND660 (Nuclear Data Inc. Schaumburg, Illinois 60196) multichannel analyser. All spectral data were stored on floppy discs for subsequent computer analysis. Raw data could be recalled for later display and visual analysis on the ND660 terminal or hard copy obtained by means of an X-Y point plotter.

Neutron Activation Analysis

Neutron activation analysis is the determination of element mass (M) by quantitative measurement of the characteristic radionuclides induced by neutron irradiation (Rakovic 1970). During irradiation the activity of a radionuclide increases exponentially towards a saturation level (mA_{sat}) with increasing irradiation time (t_{irr}). This saturation level depends on the neutron cross section (σ) for the nuclear reaction, the molecular weight (M) and isotopic abundance (θ) of the target nucleus, and the neutron flux (ϕ):

$$A_{sat}=6.02\times10^{23}\times \frac{\sigma\times\theta\times\phi}{M} \qquad (1)$$

Upon removal from the reactor the induced activity decays in the usual fashion with characteristic decay constant λ ($\lambda=\ln 2/T_{\frac{1}{2}}$ where $T_{\frac{1}{2}}$ is the half life) as the cool time (t_{cool}) increases. The growth and subsequent decay of the activity is given by:

$$A=mA_{sat}\,(1-e^{-\lambda t_{irr}})e^{-\lambda t_{cool}}\ \text{dps.} \qquad (2)$$

In INAA, the gamma ray emission accompanying the decay of the radionuclide is usually measured. Allowing for detection efficiency (ε) and gamma yield (Y), the detected events accumulated after counting for time t_{count}, is given by:

$$C=\frac{\varepsilon Y m A_{sat}}{\lambda}\,(1-e^{-\lambda t_{irr}})e^{-\lambda t_{cool}}(1-e^{-\lambda t_{count}})\ \text{counts} \qquad (3)$$

Certain of the variables in the relation such as t_{irr}, t_{cool} and t_{count} are under the analysts control and may be manipulated to enhance the determination of the elements of interest. Others such as Y, θ, σ, M and λ are characteristic of the specific radionuclide and nuclear reaction by which it was produced. The available neutron flux and the absolute detection efficiency, which varies with gamma ray energy, are instrumental limitations to the technique. Certain instrumental phenomena such as pulse pile up effects which occur at relatively high count rates can be corrected for by an experimentally determined factor (In the procedures

reported here this factor is unity).

Both the precision and sensitivity with which a given radionuclide can be measured depends not only on the counts (C) observed for its characteristic gamma ray but also on the background (B) obtained in the same energy window (Currie 1968). This is expressed by a standard deviation $S=\sqrt{C+2B}$ for a single counting period. It is important to note that a major contribution to background counts can come from other activated species in the sample. By recognising the source of these contributions, one can choose t_{irr}, t_{cool}, t_{count} and the neutron flux to optimise the measurement of the elements of interest.

D. RESULTS AND DISCUSSION

Figure I shows typical INAA spectra of unlabelled feed and fecal materials following a short irradiation (45 s) at $10^{12} ncm^{-2}s^{-1}$. Gamma rays from the shortlived radionuclides of Sc, O, Al, V, Cu, Ca, Mg, U, Cl, Mn and Na in the samples can be readily observed. The most active component in both feed and fecal materials for this short irradiation is ^{28}Al and the Compton plateau associated with its 1778 kev gamma ray forms the major contribution to the background at energies below 1555 kev. The gamma emissions of ^{165m}Dy fall in this range but, as will be shown later, the background is low enough to not appreciably disturb the measurements.

A longer irradiation time increases the activity of the

longer lived radionuclides (Equation 2). Following a 2 h irradiation and 2 h cool time at 10^{12}ncm^{-2}sec^{-1}, ^{38}Cl ($T_{1/2}$=37 min), ^{56}Mn ($T_{1/2}$=2.58 h) and ^{24}Na ($T_{1/2}$=14.9 h) are very active and completely dominate the gamma spectrum (Fig. 2). Figure 2 exhibits spectral distortion (peak broadening) due to very high count rates and would be unsuitable for quantitative measurements.

After a one day cooling period (Fig 3) ^{24}Na remains intensely active. Both ^{69}Zn ($T_{1/2}$=13.8 h) and ^{42}K ($T_{1/2}$=12.4 h) have half-lives comparable to ^{24}Na but, because present in large quantities, can be seen clearly. The measurement of other elements in trace amounts is severely limited by the ^{24}Na gamma lines and their associated Compton plateaus. Even after 5 days (ca. 8 half lives) when the ^{24}Na activity has dropped to 0.4% of its original value, the quantitative measurement of other elements is still hampered by ^{24}Na (Fig 4).

The measurement of markers with half lives comparable to ^{24}Na thus presents special problems. Radiochemical procedures (De Soete et al. 1972) can be used to remove this interference but at a cost of considerable increase in complexity compared to purely instrumental methods.

As with dairy cattle rumen content (Hartnell & Satter 1979a; 1979b), the irradiated swine samples must be cooled for at least 10 days before measurement of the longer lived radionuclides such as ^{51}Cr and ^{141}Ce. In addition to the Cr and Ce markers, the natural constituents Sc, Fe and Zn can

be seen in both feed and fecal material following a 2 wk cool period (Fig 5).

Natural background radiation from the building construction materials makes up most of the background in the long cooling period spectrum (Fig 5). This can be considerably reduced by shielding of the detector. Because the sample spectrum is relatively simple, counting can also be carried out with a NaI(Tl) well detector thereby increasing the sensitivity for the long lived markers. More importantly, automated counting systems are readily available with NaI(Tl) detectors.

Dysprosium marker analysis

Two radionuclides of dysprosium can be used for INAA marker analysis; the nuclear data for both is given in Table 2. From the above it is apparent that the lowest background interference will be obtained with the shorter lived isotope ^{165m}Dy ($T_{\frac{1}{2}}$=1.26 min).

An analysis scheme of t_{irr}=45 s, t_{cool}=30 s, and t_{count}=60 s at a source to detector distance of 3 cm was used. At the level of marker inclusion used (25 ppm), acceptable ^{165m}Dy counting statistics were obtained with a reduced reactor flux of 1 x $10^{11}ncm^{-2}sec^{-1}$. This lower flux reduces the induced background activity as well as ^{165m}Dy by a factor of 0.1 (Equations 1 and 2). Figure 6 shows a typical spectrum of a labelled feed sample under the standard conditions of marker analysis. The reference pulser

peak appearing at channel 2000, electronically simulating a detected gamma ray but with nonrandom input rate, can be used to make pulse pileup corrections. Since the busy time was never allowed to exceed 10%, these corrections were not required.

While extending the time of irradiation would increase the ^{165m}Dy activity (Equation 3) it also results in increased background activity due to ^{28}Al. Where a major background component has a similar half-life to the element of interest the increased activity due to longer irradiation may be offset by concurrent changes in background activity. A similar argument may be applied to the quantitative measurement of the longer lived ^{165}Dy ($T_{\frac{1}{2}}$=2.33 h). Although the short lived ^{28}Al may be allowed to decay away, any attempt to increase the irradiation times to increase ^{165}Dy activity will result in proportionally increased ^{56}Mn, ^{38}Cl and ^{24}Na activities which, as was shown earlier, will adversely influence the ^{165}Dy measurement.

Nevertheless, Young et al. (1975) used the longer lived ^{165}Dy when studying ruminant digesta. In contrast, Ellis (1968) working with ^{165m}Dy and ruminants fed a hay diet found pre-irradiation chemistry (wet ashing) necessary to reduce the ^{24}Na activity to manageable levels. While elemental Dy levels of 25 ppm were used in the present investigation the method allows considerable flexibility in Dy inclusion levels. The determination and detection limits (Currie 1968) for Dy with the above method are 2.5 ppm and

0.73 ppm respectively for 100mg samples.

A distinct advantage of the present analysis scheme is the speed of analysis. An INAA throughput of 45 samples/h has been routinely achieved and, as no chemical procedures are involved, sample preparation is minimal. This advantage comes, in part, from the inherent characteristics of the SLOWPOKE reactor. Accurate presettable irradiation and short transfer times (1.5 s) are achieved with the integral pneumatic rabbit system. The very stable, uniform and reproducible neutron flux of the SLOWPOKE reactor alleviates the need for time consuming, repetitive comparator standards and flux monitors (Ellis 1968) or for corrections such as the tier effect reported by Young el al. (1975). Because correction factors for geometry and flux are not required, digestibility coefficients can be calculated by a simple ratio of detected counts in feed and digesta. The earlier quoted coefficient of variation bears testimony to the precision available with the instrumentation.

Dysprosium is relatively inexpensive and can be readily included in animal feed to provide a rapid means of evaluating the digestibility of feedstuffs on a commercial scale. Digestibility coefficients could therefore be made available to producers on the basis of grab samples from feed and fecal material (Kennelly et al. 1980).

<u>Cerium and Chromium marker analysis</u>

Of the two chromium radionuclides produced by thermal neutron irradiation, only the 28 day ^{51}Cr is available for INAA by gamma spectroscopy. Of the several cerium products, both ^{139}Ce ($T_{1/2}$=33 h) and ^{141}Ce ($T_{1/2}$=32.6 d) have been used for INAA (Tolgyessy and Varga 1974). In the swine digesta of this study, however, the short lived ^{139}Ce cannot be detected against the high ^{24}Na background at the level of inclusion (61 ppm) used.

Both ^{141}Ce and ^{51}Cr are best observed after a cooling period of 2-3 weeks when the majority of the sample activity has decayed to negligible levels. The analysis scheme employed for Cr and Ce marker measurements was: t_{irr}=2 h at $10^{12}ncm^{-2}sec^{-1}$, t_{cool}=10-18 days and t_{count}=20 minute at a sample to detector distance of 3 cm. At the levels of marker addition employed, this gave adequate counting statistics for both the 320 kev ^{51}Cr and the 145 kev ^{141}Ce gamma lines for feed samples. In the case of Ce, counting statistics can be improved by increasing inclusion levels to 100-200 ppm. The determination limit (Currie 1968) for Cr and Ce for the INAA scheme outlined here has been calculated as 204 and 173 ppm respectively for 100mg samples. The detection limits were found to be 64 ppm and 56 ppm respectively.

Longer irradiation and counting times would give lower limits of quantitation but would result in increased analysis time. A better approach would be the use of a

NaI(Tl) well detector which, at the cost of reduced resolution, provides higher detection efficiency and is amenable to automation. Because of the long irradiation time required for ^{51}Cr and ^{141}Ce their use in routine analysis can be optimised by batch irradiations. The simultaneous irradiation of several hundred samples is possible with the SLOWPOKE . Such a system would make the use of ^{51}Cr and ^{141}Ce , as determined by INAA, more attractive.

In summary, quantitative analysis procedures for the use of Dy, Ce and Cr as inert markers for animal feed and digesta samples using INAA in the SLOWPOKE reactor have been investigated. In addition, a study of the major induced background activities in feed and digesta samples indicates greatest success can be expected for markers whose radioisotopes have either very short halflives ($T_{1/2}$ < several min) or moderately long half lives ($T_{1/2}$>> several days). This finding bears particularly on the potential for routine analysis where high sample throughput is required. Using the short lived ^{165m}Dy and on-line analysis with a Ge(Li) detector, sample throughput of 45/h is easily achieved. For the longer lived ^{51}Cr and ^{14}Ce , batch irradiations and automated counting with a well detector can provide high sampling capability but analysis time is extended over several weeks.

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Table 1. Experimental Diet+

Ingredient (%)	
Barley	50.0
Wheat	31.5
Soybean meal	15.0
Iodized salt	0.5
Calcium phosphate	1.0
Calcium carbonate	1.0
Vitamin-mineral premix *	0.6
Chromic oxide	0.4
	100.0

* Contributed the following per kilogram of diet: Zn, 120 mg; Cu, 10 mg; Mn, 48 mg; Fe, 100 mg; Se, 0.1 mg; Vit. A, 7,500 IU; vitamin D, 700 IU; vitamin E, 45 IU; riboflavin, 12 mg; niacin, 40 mg; calcium pantothenate, 27mg vitamin B_{12}, 28μg

+ Containing 25 and 61 ppm elemental dysprosium and cerium respectively

Table 2. Nuclear data+ for dysprosium, chromium, cerium and the major elements which contribute to background activity

Element	Target Isotopic Abundance (%)	Radio - Nuclide	Half Life	Saturation Activity *	Strongest gamma Line Kev‡	Other Lines Kev‡
Dy	28.1	Dy^{165m}	1.26 min.	1.85×10^5	108.2 (3%)	XRays 45.7 ; 52.4 515.5(1.5%) 361.7(.534%) 153.7(0.24%) 96.0(.04%)
		Dy^{165}	2.33 h	2.77×10^5	94.7 (3.7%)	XRays 47.3 ; 54.2 361.7(.87%) 633.4(.59%) + others
Cr	4.35	Cr^{51}	27.7 day	8.19×10^2	320.1(10%)	None
	2.36	Cr^{55}	3.52 min.	.38	negligible	None
Ce	88.5	Ce^{141}	32.6 day	2.12×10^2	145.4(48%)	XRays 35.9 ; 41.0
	11.1	Ce^{143}	33.0 h	44.4	293.3(42%)	57.4(12.1%)664.6(5.4%) 722.0(5.2%) + others
Al	100	Al^{28}	2.24 min.	4.95×10^2	1777.8(100%)	None
Mn	100	Mn^{56}	2.58 h	1.43×10^4	846.8(99%)	1810.7(27%) 2113.1(14%) & others
Na	100	Na^{24}	15.03 h	1.33×10^3	1368.5(100%)	2754.1(100%)
Cl	24	Cl^{38}	37.3 min.	.4336	2167.6(47%)	1642.4(32%)

+ Table of the isotopes 7th Edition Edited by C.M. Lederer & V.S. Shirley. John Wiley & Sons(1978).

* For a thermal flux of 10^{12} ncm^{-2} Sec^{-1} and a sample mass of 1 μg.

‡ Values in brackets refer to gamma yield.

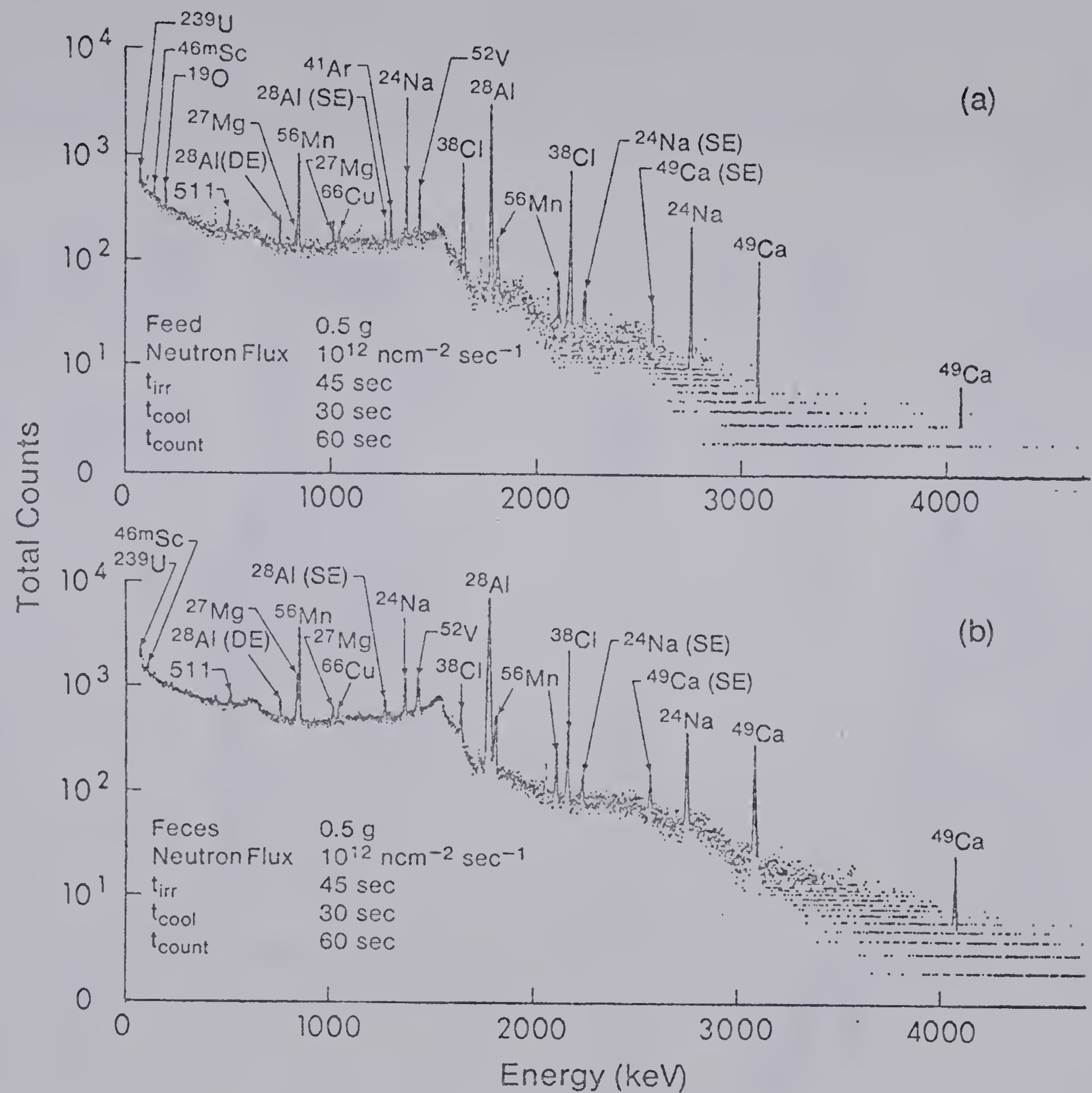


Fig. 1 Spectra of unlabelled feed (a) and fecal (b) material following a 45 s irradiation and 30 s cool time. Unlabelled peaks are due to natural background.

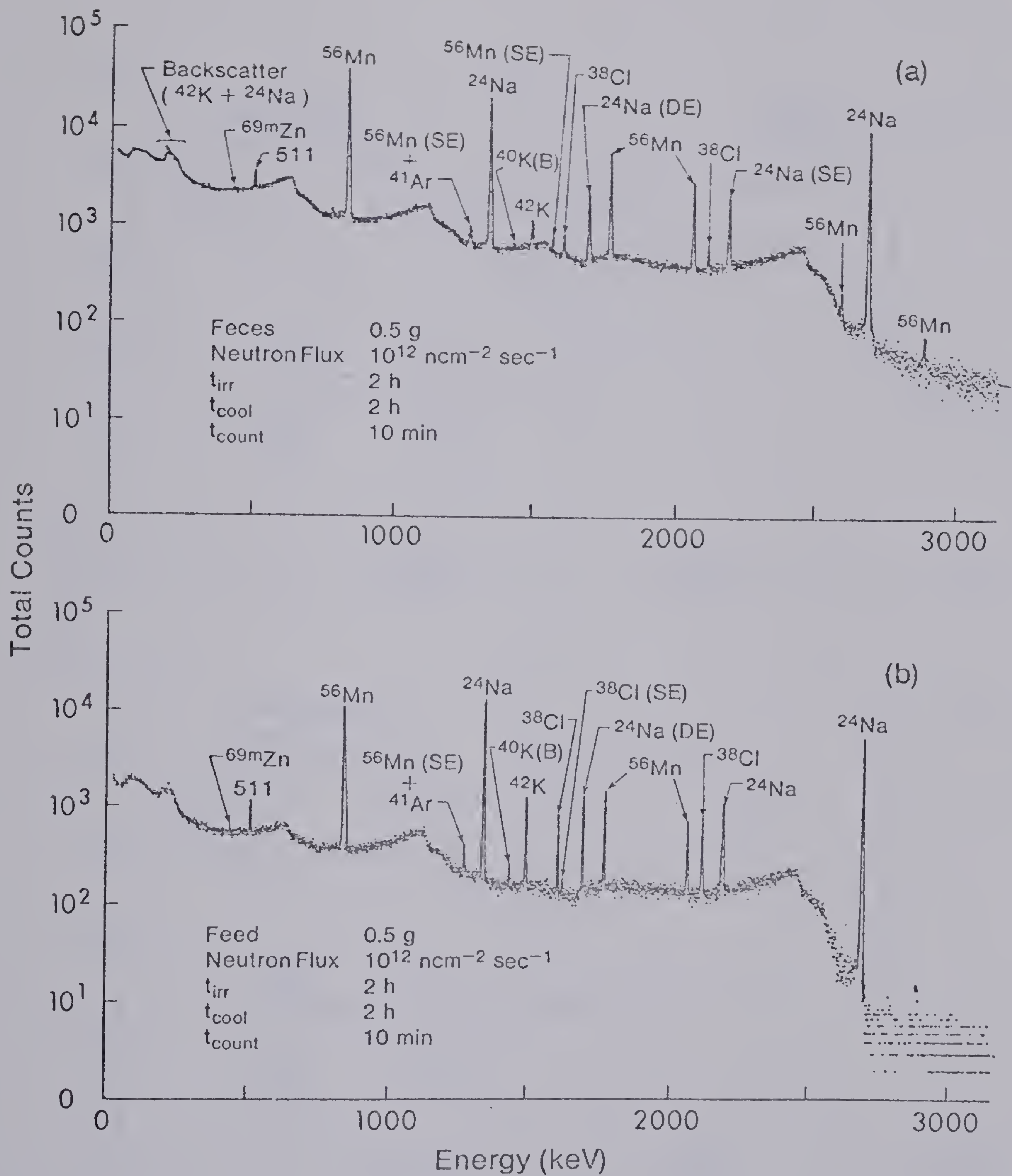


Fig. 2 Spectra of unlabelled fecal (a) and feed (b) material following a 2h irradiation and 2h cool time.
Unlabelled peaks are due to natural background.

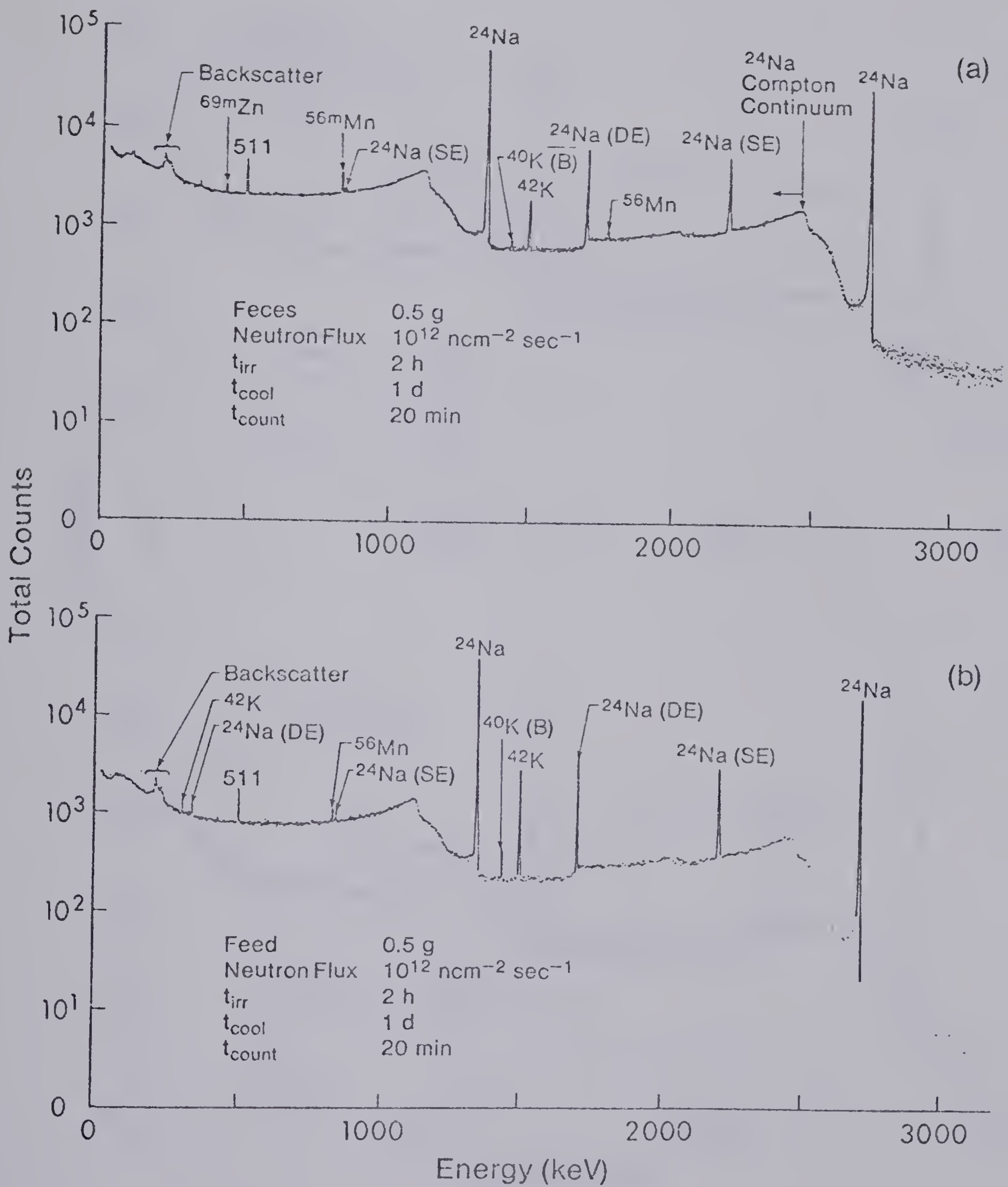


Fig. 3 Spectra of unlabelled fecal (a) and feed (b) material following a 2h irradiation and 1d cool time.
Unlabelled peaks are due to natural background.

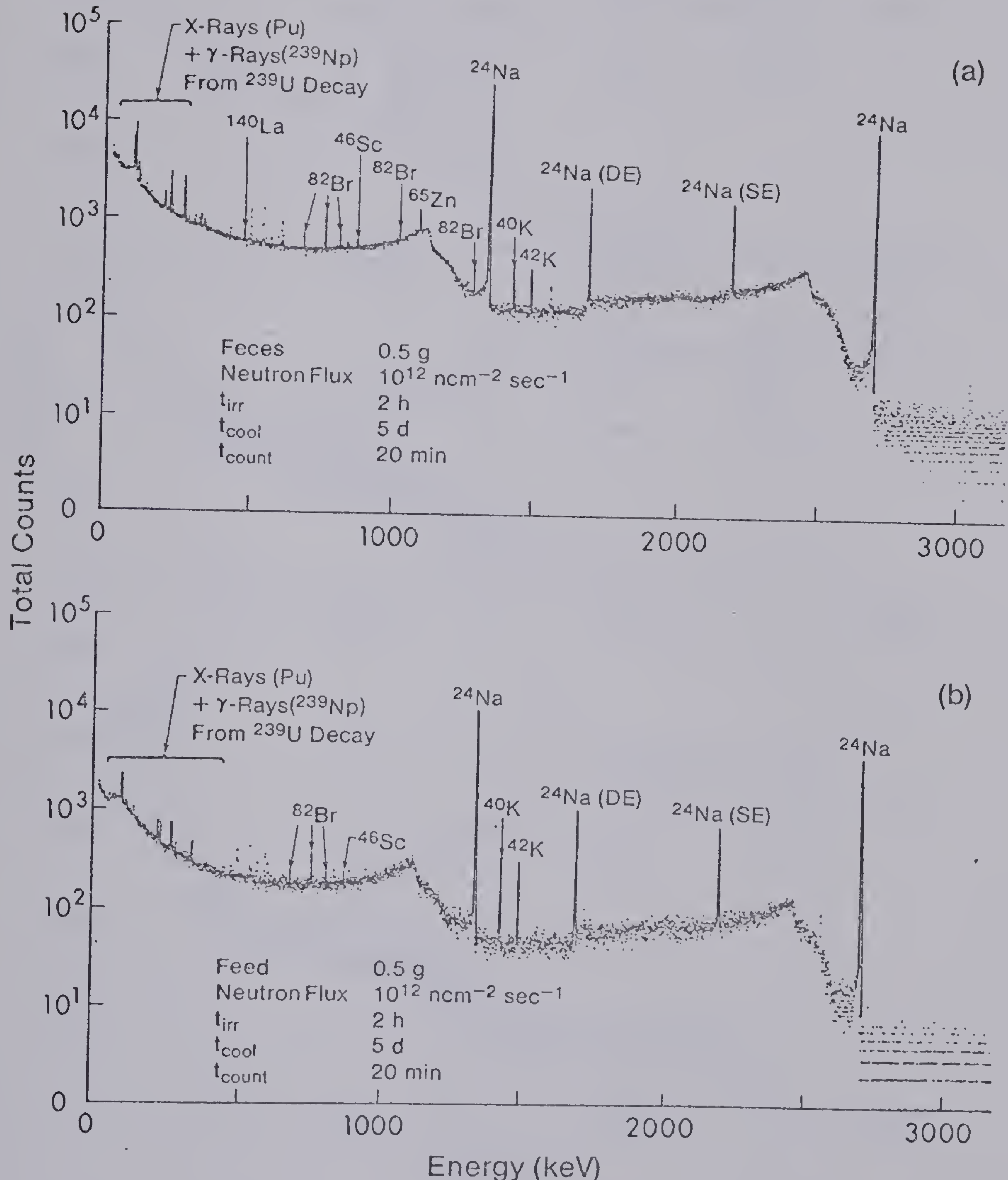


Fig.4 Spectra of unlabelled fecal (a) and feed (b) material following a 2h irradiation and 5d cool time.
Unlabelled peaks are due to natural background.

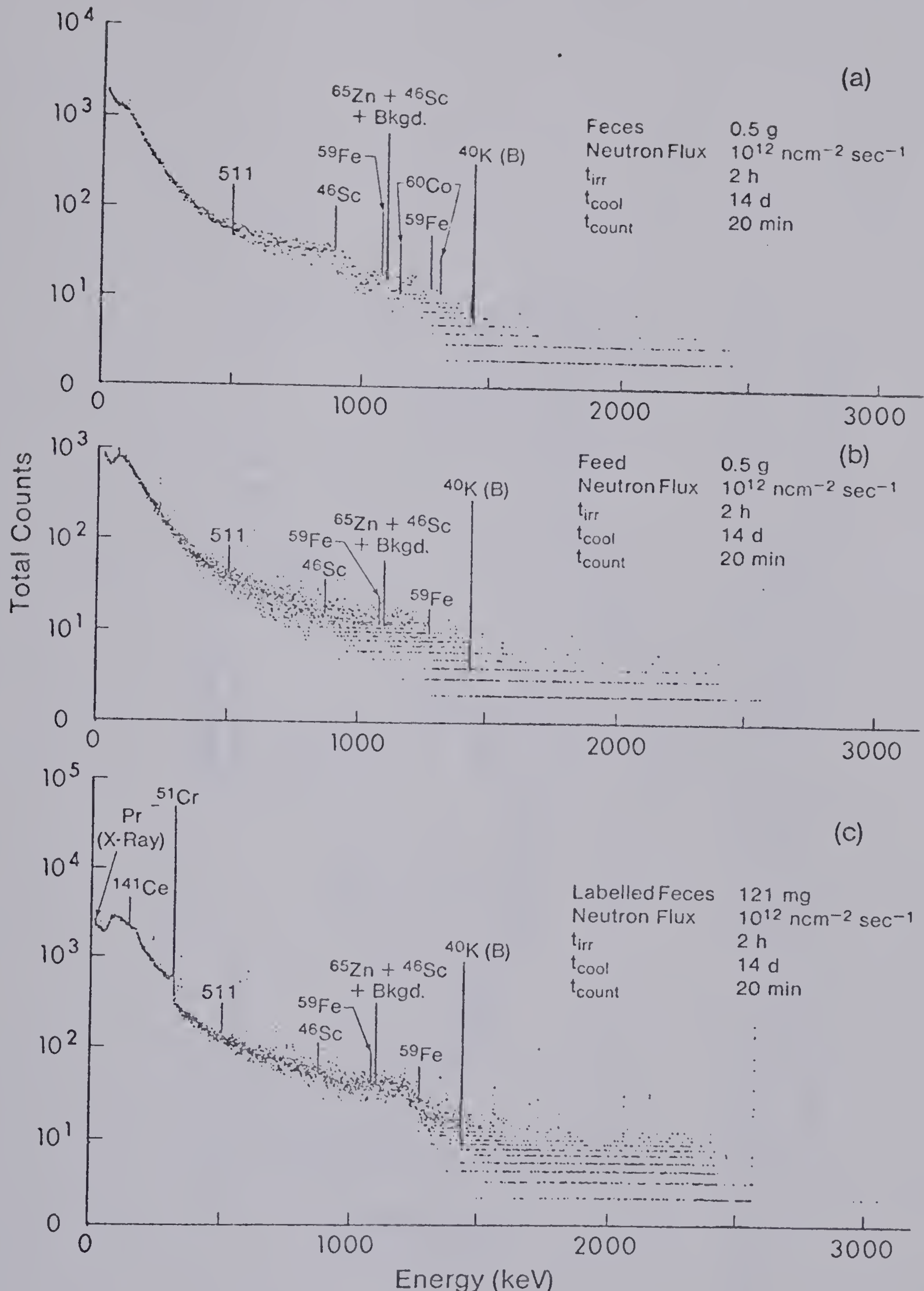
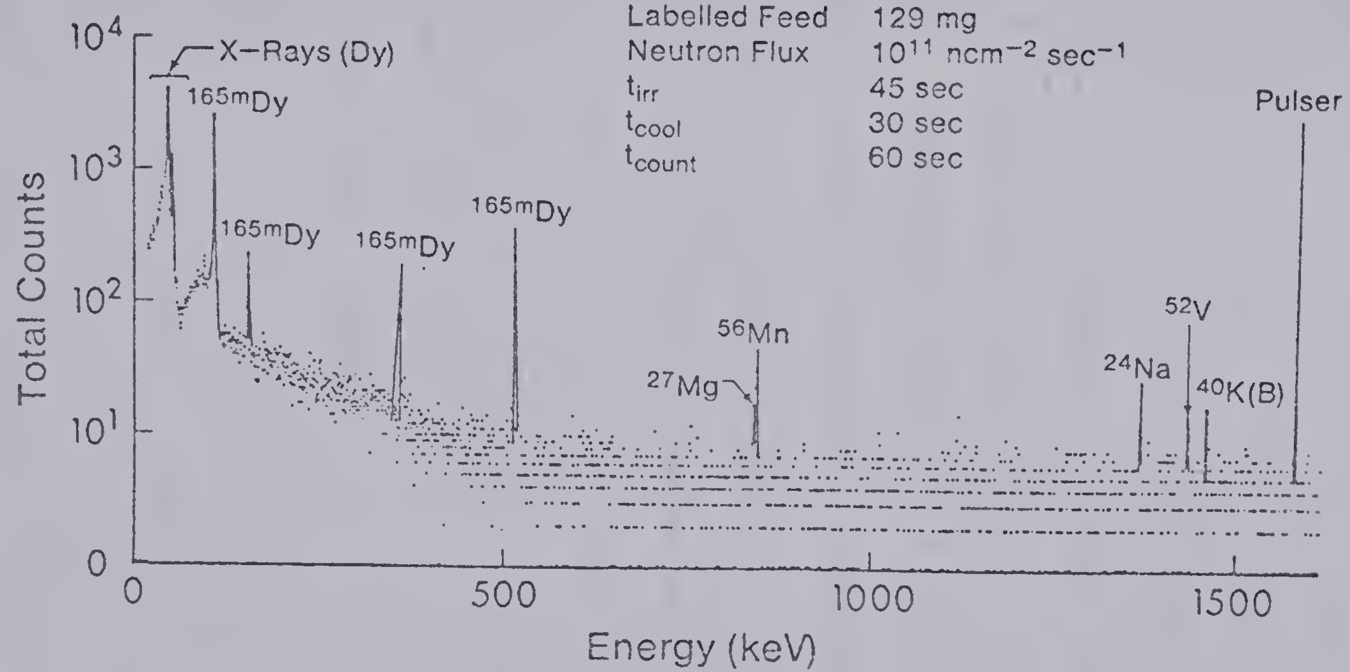


Fig. 5 Spectra of unlabelled fecal (a) and feed (b) and labelled (c) (Ce and Cr) fecal material following a 2h irradiation and 14d cool time. Unlabelled peaks are due to natural background.



<u>Fig. 6</u> Spectrum of labelled(Dy) feed material following a 45 s irradiation and 30 s cool time.
Unlabelled peaks are due to natural background.

VIII. APPENDIX II. DYSPROSIUM AS AN INERT MARKER IN SWINE DIGESTIBILITY STUDIES

A. ABSTRACT

The feasibility of using dysprosium as an inert marker for digestibility studies with swine has been investigated. Eight barrows and 8 gilts of initial weight averaging 65 kg were fed four different diets with crude fiber levels ranging from 4.1 to 10.2%. Dysprosium was included in the diets at 25 ppm and the induced radioactivity in feed and fecal samples, following irradiation in the Canadian SLOWPOKE reactor, was measured with a Ge(Li) detector coupled to a multichannel analyzer. Dysprosium was evenly distributed in feed and fecal samples. Ingested dysprosium was quantitatively recovered in feces and there was no evidence of variation in its daily excretion following a 7 day adaptation period. Dry matter and crude protein digestibility coefficients obtained with the dysprosium ratio technique were not significantly different from those determined by means of total fecal collection. The present results indicate that dysprosium, as determined by instrumental neutron activation analysis, can be used as a reliable indicator in swine digestibility studies.

B. INTRODUCTION

The rare earth elements are becoming increasingly popular as markers in ruminant studies (Gray and Vogt 1974; Luckey et al 1975). Among the elements which have been studied are cerium (Ce) (Huston and Ellis 1968; Miller et al 1967), and dysprosium (Dy) (Ellis 1968; Young et al 1975). These elements are not absorbed from the gastrointestinal tract and possess many of the properties of ideal nutritional markers (Ellis 1968). Radioactive isotopes of these elements have been used but result in animal contamination and problems associated with the disposal of large quantities of radioactive waste. The use of instrumental neutron activation analysis (INAA) circumvents both these difficulties.

Ellis (1968) wet ashed fecal and rumen samples prior to INAA because irradiation of unprocessed samples was not possible under the experimental conditions employed. However, Young et al (1975) successfully irradiated similar samples without prior processing. In both these studies the flux characteristics of the reactors employed necessitated the use of simultaneous internal standards with correction factors for each sample. In contrast the inherent stability, uniformity and reproducibility of the neutron flux is a design feature of the Canadian SLOWPOKE reactor (Jervis et al 1977) and obviates the need for repeated standards and corrections for variation in neutron flux.

The purpose of this study was to determine the

reliability of Dy as an inert marker in swine digestibility studies using the SLOWPOKE reactor and INAA.

C. MATERIALS AND METHODS

Sixteen crossbred pigs (Yorkshire x Lacombe, 8 barrows and 8 gilts) with average initial and final weights of 65 and 75 kg respectively, were confined to stainless steel metabolism crates in a room maintained at approximately 20°C. At 0800 h and 1600 h daily, the pigs were fed 0.7 and 1.4 kg respectively, of the diets shown in Table 1. The control diet (diet 1) was a standard University of Alberta growing-finishing ration with 4.1% crude fiber. Diets 2, 3 and 4 each contained 22% oat hulls resulting in an analyzed crude fiber level of approximately 10%. Dysprosium chloride ($DyCl_3 6H_2O$) was incorporated into the feed at a concentration of 25.4 ppm elemental Dy. It was first added to 100g of a feed ingredient ground to a similar fineness; the rest of the feed was then added gradually until complete mixing of Dy with the experimental diet was achieved.

The animals were allowed 7 days to adjust to the feed, feeding regime and metabolism crates before starting a 7-day total fecal collection period. The 4 experimental diets were sampled as fed and composite samples were ground through a 20 mesh screen before analysis. Daily fecal collections from each animal were thoroughly mixed and three 50g subsamples taken for subsequent freeze-drying and analysis by INAA. The

remainder of the composite fecal samples were dried in a forced air oven at 60°C for 3 days to determine dry matter content. Crude protein content (AOAC 1975) was determined on the composite daily fecal collection from each pig.

Grab samples of fresh feces (50g) were taken twice daily (0800-1200 and 1200-1600 h) and freeze-dried without mixing. Five 100 mg samples from each freeze-dried grab sample and daily subsample was accurately weighed into acid cleaned $1.5 cm^3$ polyethylene vials and irradiated in the SLOWPOKE reactor. Briefly, the activation scheme consisted of irradiation for 45 sec at a thermal neutron flux of 1×10^{11} $ncm^{-2}sec^{-1}$, cooling for 30 sec and counting the photopeaks of ^{165m}Dy for 60 sec with a Ge(Li) detector. This activation scheme enabled samples to be processed , and the spectral data stored on floppy discs, at the rate of 30-45 samples per hour. The gamma ray spectroscopy system consisted of a solid state Ge(Li) coaxial detector (18.5% relative efficiency and 1.93 Kev FWHM at 1332 Kev) coupled to a 4096 channel ND660 (Nuclear Data Inc.) multichannel analyser. All raw data could be recalled for later analysis using the ND660 computer system to quantitatively determine the spectral peaks associated with ^{165}Dy.

Data were analyzed using least squares analysis of variance for unequal numbers (Harvey 1960) adjusting for all identified sources of variation (Mehlenbacher 1978). Means for significant treatment differences were compared using Student-Newman-Keuls multiple range test (Steel and Torrie

1960) with adjustments for unequal number of observations per mean.

D. RESULTS AND DISCUSSION

Dysprosium (^{165m}Dy) decays with the emission of 4 single gamma rays at 108, 154, 362 and 516 KeV . Dry matter digestibility coefficients determined by total collection and ^{165m}Dy ratio technique, for each photopeak, are presented in Table 2. No significant differences were observed between dry matter digestibility coefficients obtained by total collection and that obtained using any or all of the 4 photopeaks of ^{165m}Dy.

Grinding feed samples to a flour improved the homogeneity of Dy distribution, with the coefficient of variation (CV) among 10 replicate 100mg samples decreasing from 2.7 to 1.5 percent. The lower CV is probably a good reflection of the variation associated with the analytical technique; the difference in the two CV representing homogeneity variation in the coarse ground feed. Factors contributing to the analytical variation include uncertainties of sample weighing, neutron flux, counting geometry and statistical counting errors.

Excellent homogeneity for Dy distribution was observed in samples from composite daily fecal collections (Table 3). The coefficient of variation among 10 replicate 100mg unground fecal samples was found to be 2.1%. Grinding of

fecal samples to various degrees of fineness did not alter the homogeneity observed. While the CV observed for Dy concentration in fecal samples is greater than that deduced for the analytical method, it is less than that observed with unground feed samples indicating uniform distribution of Dy in fecal material.

In a study with ruminants Huston and Ellis (1968) showed that cerium was rapidly adsorbed on and remained tenaciously bound to digesta particles. Similar results have been observed for Dy (Ellis 1968), samarium and lanthanum (Hartnell and Satter 1979a; 1979b). However, it is important when sampling to select material of representative particle size as particle size may affect the extent of adsorption of Dy. Where samples are mixed wet and freeze-dried, separation of digesta does not occur, and uniform sampling is possible. Under such circumstances grinding of fecal material is unlikely to lead to greater homogeneity in Dy distribution.

Dry matter digestibility (DMD) coefficients were similar irrespective of the day of sampling (Table 4). The absence of significant daily variation indicates that DMD can be reliably determined by taking subsamples from daily composite samples any day following a 7-day adaptation period. These results are supported by those of Ellis (1968) who found low within and between day variation in fecal Dy concentration of cattle. Olbrich et al (1971) have reported similar results when using cerium as a marker in ruminants.

A comparison of digestion coefficients based on a

single morning (0800-1200 h) and afternoon (1200-1600 h) sample from one pig on each diet is shown in Table 5. While not significant, higher digestion coefficients were consistently obtained from morning sampling which could be associated with longer digesta retention time in the animal or the larger feed allowance at 1600h.

One of the most important features of Dy as a marker is its use to accurately determine digestion coefficients using grab samples taken twice daily (Table 6). No significant differences were observed in DMD coefficients obtained from total collection and those determined from a composite of a single morning and afternoon grab sample from each animal.

As with DMD, no significant differences in crude protein digestibility coefficients (Table 7) were observed using the total collection or ^{165m}Dy ratio method. However the digestion coefficients observed for the total collection method tended to be slightly higher.

The INAA of urine samples from animals fitted with urinary (bladder) catheters failed to detect Dy. The quantitative recovery (100±2%) of ingested dysprosium in fecal samples, observed in this study, is in agreement with Hutcheson et al (1975) and Ellis (1968) who concluded that dysprosium was essentially unabsorbed from the intestinal tract of ruminants. In contrast Luckey et al (1977; 1979) observed , that in humans, recovery ranged between 73 and 97% for various rare earth elements. While these measurements followed single pulse rather than continuous

marker ingestion, it is unlikely that this could account for the low recoveries reported.

The ^{165m}Dy ratio technique has been shown to give digestibility coefficients similar to those obtained with total collection. The results indicate that Dy can be used as a reliable indicator in swine digestibility studies. The use of this method in digestibility studies for swine has many advantages over conventional methods of measuring digestibility including speed, simplicity and applicability to on farm digestibility studies.

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Table 1. Composition (%) and chemical analysis of experimental diets.

Ingredients (%)	Diet 1	2	3	4
Barley	50.0	35.4	39.0	40.0
Wheat	31.5	20.0	24.5	-
Soybean meal	15.0	19.1	11.7	23.0
Oat hulls	-	22.0	22.0	22.0
Iodized salt	0.5	0.5	0.4	0.5
Calcium phosphate	1.0	1.0	0.8	1.0
Calcium carbonate	1.0	1.0	0.8	1.0
Vit. mineral mix+	1.0	1.0	0.8	1.0
Tallow	-	-	-	11.5
Determined analysis (as-fed)				
Gross energy (MJ/kg)	17.10	17.00	16.80	20.70
Digestible energy (MJ/kg)	14.10	12.20	12.50	14.70
Crude Protein (%)	17.1	17.0	14.4	17.3
Crude fibre (%)	4.10	9.80	9.60	10.20
Ether extract (%)	2.13	1.97	2.02	15.60
Ash (%)	5.32	6.27	5.46	5.46

+ Contributed the following per kilogram of diet: Zn, 120 mg; Cu, 10 mg; Mn, 48 mg; Fe, 100 mg; Se, 0.1 mg; vitamin A, 7,500 IU; vitamin D, 700 IU; vitamin E, 45 IU; riboflavin, 12 mg; niacin, 40 mg; calcium pantothenate, 27 mg; vitamin B_{12}, 28 µg.

Table 2. Dry Matter Digestibility Coefficients (%) Obtained Using the 108, 154, 362 and 516 Kev Photopeaks of ^{165m}Dysprosium.

	Diet				
	1	2	3	4	SEM
Total Collection	81.4a	70.2b	72.8b	65.0c	1.3
Dy 108	80.0a+	71.3b	73.4b	66.3c	.41
Dy 154	80.0a	70.9b	74.1b	64.0c	.56
Dy 362	79.5a	72.4b	72.8b	66.8c	.49
Dy 516	80.5a	71.0b	73.5b	67.1c	.40
Mean Dy	80.1a	71.3b	73.5b	66.3c	.39

a,b,c Means in the same row or column with different letters are significantly different ($P < 0.05$)

+ Each value for Dy is the mean of 56 observations (4 animals x 7 days, duplicated)

Table 3. Dry Matter Digestibility Coefficients (%) Determined From Subsamples Taken From Feces of One Pig Fed Each Diet

Diet	Subsample A	Subsample B	Subsample C	SEM
1	82.8+	82.0	82.9	0.85
2	67.2	67.6	67.7	0.91
3	75.7	75.4	75.6	0.96
4	63.5	63.7	62.6	0.96

\+ Each value is the mean of five observations calculated using the 108 keV photopeak of ^{165m}Dy

Three 50-g subsamples (A,B and C) were taken from the composite daily collection; five 100-mg samples from each were then analyzed for Dy.

Table 4. Daily Variation in Dry Matter Digestibility Coefficients

Diet	Day 1	2	3	4	5	6	7	SEM
1	81.6a+‡	80.8a	80.3a	80.0a	81.0a	81.3a	80.5a	0.75
2	70.8b	71.4b	71.8b	71.8b	70.1b	70.9b	71.5b	0.90
3	73.5b	72.1b	73.0b	73.5b	73.7b	72.6b	72.3b	0.97
4	66.0c	65.3c	65.9c	65.5c	65.7c	66.9c	66.8c	0.97

a,b,c Means in the same row or column with different letters are significantly different ($P < 0.05$)

+ Each value is the mean of 8 observations- 2 samples per animal obtained from daily total fecal collection.

‡ Calculated using the 108 keV photopeak of ^{165m}Dy

Table 5. Comparison of Dry Matter Digestibility Coefficients (%) Obtained From the Morning and Afternoon Grab Samples From One Pig Fed Each Diet

Diet	Morning	Afternoon	SEM
1	81.6+	80.4	1.7
2	73.0	72.6	1.7
3	75.4	72.4	1.7
4	66.7	63.6	1.8

+ Each value is the mean of 7 observations (7 days) calculated using the 108 keV photopeak of ^{165m}Dy

Table 6. Dry Matter Digestibility+ Coefficients Calculated from total collection and Dysprosium using Grab Samples*

Method	Diet 1	2	3	4	SEM
Total Collection	82.1a‡	68.8b	73.5b	64.7c	2.6
Dysprosium	81.0a	71.8b	74.3b	65.1c	1.6

\+ For one pig on each treatment

* Two grab samples each of 50 g taken morning and afternoon from each animal; samples were freeze dried and 100 mg of each were analyzed.

a,b,c Means in the same row of column with different letters are significantly different ($P<0.05$).

‡ Calculated using the 108 keV photopeak of ^{165m}Dy

Table 7. Comparison of Crude Protein Digestibility Coefficients obtained by Total Collection and by Using Dysprosium as an Indicator

	Diet				
Method	1	2	3	4	SEM
Total Collection	82.0	82.7	79.1	81.7	1.4
Dysprosium	81.2+	81.6	78.9	80.8	0.3

\+ Each value for Dy is the mean of 56 observations (see Table 2) calculated using the 108 keV photopeak of ^{165m}Dy